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COLLECTED PAPERS

(PART I.)

FROM THE

RESEARCH LABORATORIES

OF THE

ROYAL COLLEGE OF PHYSICIANS, LONDON,

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EDITED BY

T. G. BRODIE, M.D.,

(Director of the Laboratories).

1903.

This volume of Collected Papers contains reprints of most of the papers that have appeared from these laboratories during the past four years. The first paper, that by Dr. Pavy, is unfortunately only an abstract of the full paper, since the type was broken up before the necessary number of reprints had been asked for.

The last four papers are reprints of work published from the Physiological Laboratory, St. Thomas's Hospital Medical School, which appeared during my tenure of the Lectureship of Physiology at St. Thomas's.

T. G. BRODIE.

April, 1903.

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An Enquiry into the Effects on the Blood and Urine of the Intravenous Injection of various Carbohydrates standing in relation to Animal Life.

By F. W. PAVY, M.D., LL.D., F.R.S.

(*From the Laboratories of the Royal Colleges of Physicians and Surgeons, London.*)

ABSTRACT.

[The communication was published in the *Journal of Physiology*, vol. xxiv., No. 6, pp. 479-517, August 21, 1899, where the reader is referred for details. The abstract has been prepared by the author for this collection of papers in default of copies of the communication having been secured.]

HISTORICAL.

A *résumé* of the literature of the subject is first given.

PROCEDURE FOR THE COLLECTION OF BLOOD.

In the collection of the blood, as elsewhere pointed out, it is necessary to proceed with the utmost expedition in order that no change may take place in the contents of the circulation from the *post-mortem* production of sugar in the liver. The plan adopted was to pith and instantly afterwards thrust a pair of scissors into and lay open the chest and snip up the heart. With an assistant on each side of the table, having hold respectively of the hind and fore legs, the animal was turned over rapidly after pithing for the opening of the chest, and then turned back again over an evaporating basin standing at hand for the reception of the blood, which was at once stirred to defibrinate.

The time occupied in pithing and collecting the blood in the basin, taken by the chronograph, was on an average about fifteen seconds.

DETERMINATION OF SUGAR.

Alcoholic extraction was as a rule employed, and the sugar was determined by titration with the ammoniated cupric solution before and after hydrolysis by boiling with an acid. The steps of the process are described in full. K represents the cupric oxide reducing power existing before, as compared with that found after hydrolysis and taken as 100.

NORMAL CONDITION OF BLOOD AND URINE.

Examples are given showing the figures obtained in each instance.

In the case of the blood, the mean of 12 observations in the rabbit gives 1·089 of sugar per 1000 before hydrolysis and 1·495 after, K = 73 ; while that of 6 observations in the cat is 0·880 per 1000 before and 1·140 after, K = 77.

In the case of the urine, the mean of 16 observations in the rabbit was 3·69 per 1000 before and 7·03 after, K = 52 ; and that of 12 observations in the cat 2·75 before, and 5·87 after, K = 46. One observation in the dog gave 4·07 per 1000 before and 6·36 after hydrolysis, K = 64.

INTRAVENOUS INJECTION.

The carbohydrates used were saccharose, maltose, lactose, galactose, lævulose, dextrose, and glycogen. Full details are given in the paper and the results are all expressed in tabular form. 130 experiments were made, the animal used being invariably a rabbit. In every case 10 cc. of sugar solution per kilo of body weight were injected into the vein of the ear. The sugar solutions contained 40, 10 or 2·5 grms. per 100 cc.

A general review of the results is next given.

Blood.—The mean amounts of sugar found in the blood instantly after the completion, that is within 1½ minutes of the commencement of the injection of 4 grms. per kilo body weight, were as follows :—

Saccharose	Maltose	Lactose	Galactose	Lævulose	Dextrose
22·91	18·03	18·45	12·05	15·95	14·86

A calculation of the amount of sugar that would be present in the blood by simple addition of 10 cc. of 40 per cent. solution of sugar to the 70 cc. of blood which are usually considered to be present for each kilo weight of rabbit, gives 50 per 1000, which is very widely different from the observed quantity. It was thought that dilution of the blood by fluid drawn from the tissues, by reason of the increased tonicity of the blood, might account for the discrepancy observed, and the hæmoglobin of the blood was therefore determined before and after injections of saccharose and dextrose. It was found to fall in the first case from 100 to 53 and in the second from 100 to 63. This about explains the proportion of saccharose found, but is quite inadequate to account for the values obtained with dextrose. The mean amounts of sugar found five minutes after injection were :—

Saccharose	Maltose	Lactose	Galactose	Lævulose	Dextrose
14·42	11·04	10·41	10·37	10·33	10·46

fifteen minutes after injection,

11·94	8·05	7·25	8·33	7·36	8·17

After injection of 1 grm. per kilo the amounts found "instantly" were :—

Saccharose	Maltose	Lactose	Galactose	Lævulose	Dextrose
6·04	6·43	6·12	6·14	6·48	6·12

The quantity is in every case approximately half that which would be given by adding 10 cc. of 10 per cent. sugar solution to 70 cc. of blood.

The quantity of sugar present gradually falls, until at the end of an hour the figures obtained for dextrose and lævulose are sensibly normal (1·33—1·44 per 1000), while those for galactose, maltose and saccharose are somewhat higher (1·7—1·9 per 1000), and those for lactose are highest (2·33 per 1000).

After injection of 0·25 grm. per kilo the amounts found "instantly" were :—

Saccharose	Dextrose
2·55	2·66

Half an hour after the injection of 2·5 per kilo of dextrose, the sugar contents of the blood were found to be normal, but after another half hour the amount was decidedly greater (mean of 5 expers., 1·85 per 1000). No explanation of this can be suggested.

Urine.—The sugar makes its way extremely rapidly into the urine, as much as 4·5 per cent. of the saccharose injected finding its way out within 1½ minutes. The percentage excreted within one hour of the injection of 1 grm. per kilo was:—

Saccharose	Maltose	Lactose	Galactose	Lævulose	Dextrose
81·0	56·5	48·7	28·9	20·9	15·6

The figures obtained in different experiments with the disaccharids do not deviate much from the mean, whereas with the monosaccharids the deviation in individual experiments is very great. The urine secreted during one hour succeeding an injection of 0·25 grm. per kilo of dextrose was examined in 5 cases, and the mean sugar content is just double that of the 16 normal urines tabulated in the early part of the communication. Saccharose, lactose, galactose and lævulose appear to be excreted unaltered, while maltose (from the K and opticity found) seems to be to some extent hydrolysed, and dextrose gives evidence of more or less dehydrolysation having occurred.

GLYCOGEN.

Glycogen was injected only in 10 per cent. solution. The blood was found one hour afterwards to contain a mixture of carbohydrates soluble in alcohol with conspicuously low CuO reducing power.

The urine, which was always tinged with hæmoglobin and contained albumin, contained besides sugar, a considerable quantity of achroodextrins.

SUBCUTANEOUS INJECTION.

Thirty experiments were made on rabbits, using the same series of sugars as was used for intravenous injection. The sugar, 1 grm. per kilo in 10 per cent. solution, was injected from a burette into the subcutaneous tissue of the back. Blood and urine were examined in every case two hours after, and the results are tabulated in the paper.

It was found that saccharose and lactose passed rapidly into the urine, as occurred after intravenous injection. This is in harmony with the view generally taken that these sugars are incapable of being acted upon by the tissues of the body, and are eliminated as useless material.

Maltose was eliminated much less readily, and although after intravenous injection it is excreted as rapidly as lactose, after subcutaneous injection it ranks with the easily assimilable dextrose and lævulose, being less readily excreted than galactose.

General Conclusions.

The sugar normally present in the blood does not consist exclusively of dextrose. The product, in fact, given by alcoholic extraction possesses a CuO reducing power considerably below that of glucose—viz., about 75 as compared with glucose reckoned at 100.

Normal urine contains a distinctly evident and easily determinable amount of sugar which like that in the blood possesses a CuO reducing power below that of glucose. The actual CuO reducing power is considerably lower than what is found in the case of the blood.

In experiments on the intravenous and subcutaneous injection of sugars the examination of the blood as well as of the urine and the estimation of the CuO reducing effect producible before and after inversion, give an insight into the current of events beyond what is otherwise obtainable.

Whatever the explanation, the experiments show a striking difference in the amount of sugar found in the blood collected instantly after the intravenous injection of large doses (4 grms. per kilo) of the disaccharids as compared with that found after the monosaccharids. The highest figures, 21—24 per 1000, occur after saccharose. The lowest after galactose and dextrose in connection with which they stand respectively at 11—12 and 14—15 per 1000. After the injection of small doses the same disparity is not observable.

The disaccharids, saccharose and lactose seem to comport themselves within the general system as unserviceable foreign matter and become speedily cast out with the urine. They appear, as it were, to be repelled by the system and to be discharged accordingly.

The results from maltose are ambiguous. After intravenous injection they correspond with those for lactose and thus give the appearance that the two stand in an allied position—indeed the figures show a rather greater elimination of maltose than of lactose. In the experiments, however, with subcutaneous injection the elimination of maltose shows a wide difference from that of lactose and coincides instead with that noticeable after the monosaccharids, even after those with which the lowest figures occur.

After both intravenous and subcutaneous injection the results given by the monosaccharids show a low degree of elimination. Of the three, galactose distinctly escaped more freely than lævulose and dextrose; as regards the two latter, not much difference is perceptible. What there is is in the direction of higher figures for lævulose.

A notable point brought out is the alteration that dextrose undergoes. After the injection of a large quantity, on account it may be assumed of the masking influence exerted, dextrose is found in both blood and urine. Injected in less quantity, however, the initial CuO reducing action is far less than that met with after inversion. This applies alike to blood and urine, and as is noticeable with normal blood and urine the CuO reducing power expressed by K is considerably lower in the case of the urine than in that of the blood.

In contrast with dextrose no such change occurs with galactose. With lævulose the circumstances do not permit of a definite conclusion, but apparently the change does not occur.

Glycogen injected intravenously gives rise to the presence of a higher amount of sugar than normal in the blood and to the elimination of a considerable amount of low CuO reducing product in the urine.

From the contents of this communication it is learnt that with the adoption of a sufficiently delicate method of experimental procedure, the introduction of carbohydrate matter by intravenous and subcutaneous injection, in all the forms and in the smallest doses employed, told in a distinctly recognisable manner upon both blood and urine. A difference consisting of one of degree, and not of actual kind, is noticeable with the different sugars. Those which fail to be susceptible of application on introduction into the general system, are freely thrown out, but at the same time the converse does not strictly hold good, for although susceptible of application they do not miss revealing their presence in a manner distinctly to be perceived. Even with dextrose, which seems to possess the greatest aptitude for undergoing application, its introduction is made evident alike through the blood and urine, in proportion to the extent to which it is introduced. The application that occurs is not immediate but gradual, and thus evidence is obtainable of the entry that takes place by the addition occurring to the normal sugar.

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ON REFLEX CARDIAC INHIBITION.

By T. G. BRODIE, *Director of the Conjoint Laboratories of the Royal Colleges of Physicians and Surgeons, London,* AND A. E. RUSSELL, M.D., *Resident Assistant Physician, St Thomas's Hospital, London.* (Six Figures in Text.)

(*From the Conjoint Laboratories of the Royal Colleges of Physicians and Surgeons, London.*)

It has long been known that slowing or arrest of the heart can be brought about reflexly by excitation of almost any afferent nerve of the body if the stimulus be sufficiently great, and that the one nerve which can most readily produce this reflex effect is the vagus. The systematic examination of the different branches of the vagus with the direct object in view of determining which fibres play the most important part in producing the reflex has not however been carried out. Hence the experiments described in this paper have been designed.

Our experiments can be conveniently grouped for descriptive purposes in two sections, in the first of which we deal with the results obtained by electrical excitation of the vagal branches and in the second, with other modes of excitation,—chemical, mechanical, etc.—which followed as a direct consequence of the results we obtained in our first series.

Most of our experiments have been performed upon dogs, the anæsthetic employed being, as a rule, the A.C.E. mixture. In a few cases urethane was used, which was administered either subcutaneously or by intravenous injection; the dose given was 1·25 grm. per kilo of body weight. The results were precisely the same with both forms of anæsthesia. We have also confirmed our results upon cats and found the reaction the same in this animal as in the dog.

To record the effect upon the heart we usually took the carotid blood-pressure curve as being on the whole the most convenient. The records thus obtained were always checked by direct observation of the heart in those experiments in which it was exposed, by palpation, or by placing a finger upon an artery while the nerve stimulation was being carried on. We also tested whether the effects were exerted upon all four cavities of the heart uniformly, by recording the contractions of the ventricles and auricles. We found that whenever any slowing of the heart took place it was to be observed equally in both ventricles, and in auricle as well as ventricle.

I. RESULTS OF EXCITATION OF THE CENTRAL ENDS OF THE DIFFERENT BRANCHES OF THE VAGI.

A. *The vagi on the œsophagus.* These were exposed by resecting parts of the seventh and eighth ribs, artificial respiration being started. They were dissected out and loops of thread passed under them. The carotid was connected to a mercury manometer and the blood-pressure recorded. One vagus was now lifted up, and excited. This was repeated at short intervals with currents of varying strengths. The nerve was then ligatured and cut below the ligature. The central end was placed on the electrodes and stimulated as before. The results were always found to be exactly the same as those obtained from the undivided nerve. The second vagus was next treated in the same way. As a final step in the experiment the vagus was divided in the neck and its central end excited by the same strengths of currents as those already employed. In this way a direct comparison of the reaction obtainable from the two positions was obtained.

The results we obtained were very variable and were as follows:—

(*a*) Some slowing of the heart-rate accompanied by a fall in blood-pressure.

(*b*) No effect on heart-rate and a rise in blood-pressure.

(*c*) No effect on heart-rate nor on blood-pressure.

(*d*) In one instance slight slowing of the heart and a small rise in blood-pressure.

We found that inhibition was more readily obtainable by exciting these nerves in the dog than in the cat. In the latter animal the rule was that no inhibition followed, in the dog on the other hand slowing occurred in more than half the experiments. The animals which gave it were found to vary considerably in the reaction produced and in the strength of current required. As a rule strong currents were necessary[1] to produce a reaction. The results nearly resemble those obtainable by excitation of an ordinary afferent nerve. The point we wish to emphasise is, that though reflex inhibition can be commonly obtained it is never very marked, and is in no way comparable to the control reaction produced when the central end of the vagus in the neck was excited by the same strength of current. In Fig. 1 we give a blood-pressure tracing giving the record of one of these excitations which produced a positive result. A strong current was necessary and the reaction obtained was fairly good. In all cases the blood-pressure record was checked by direct observation of the heart itself.

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[1] For excitation we employed a Du-Bois coil worked by a single Leclanché battery. The electrodes used were the ordinary shielded electrodes.

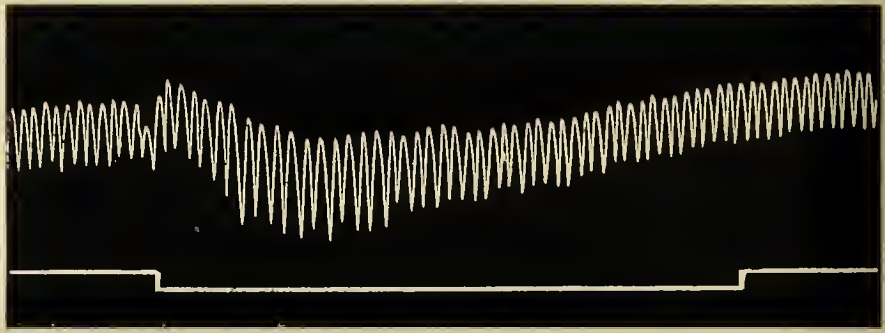

Fig. 1. Dog. Blood-pressure tracing. The zero-abscissa line has been raised. During the time indicated by the depression in this line the central end of the right vagus on the œsophagus was excited. Coil at 0.

In view of these results we did not consider it necessary to examine any lower branches of the vagi.

In connection with these results we have the experiments of Goltz[1] and others upon reflex cardiac inhibition from the stomach and intestines. In Goltz's classical "Klopfversuch" inhibition is produced in the frog by sharply striking the exposed stomach. The result of electrically exciting the nerve filaments from the viscera was not constant. Bernstein[2] showed that the afferent path for this reflex was along the sympathetic, then into the cord by fibres entering not higher than the third spinal nerve. He also showed that this was practically the same in the rabbit. Mayer and Pribram[3] also studied this reflex in mammals and found that the slowing of the heart was accompanied by a rise in blood-pressure. They found that it could be most easily brought about by suddenly distending the stomach with air, and gave experiments proving that the afferent impulses were started from the muscular or peritoneal coats and not from the mucous membrane of the stomach. Tarchanoff[1] found that the reflex was much more active if the stomach and intestines were inflamed, a result confirmed by François-Franck[2]. None of these observers have attempted to determine whether section of the vagi in the thorax materially modifies the course of this reflex, hence we performed two experiments upon cats with this particular object in view. The stomach was exposed by a small median incision through the upper part of the abdominal wall. In one experiment the inflation of the stomach was effected by passing a tube through the œsophagus, and in the other by making a small incision through the stomach wall at the pylorus. The inflation was performed in the same way as that used by Mayer and Pribram, and consisted in tying a small rubber bag to the end of a glass tube, this was then introduced into the stomach and inflated as required.

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[1] Goltz. *Virchow's Archiv*, XXVI. p. 1. 1863.

[2] Bernstein. *Archiv für Physiol.* 1864, p. 614.

[3] Mayer and Pribram. *Sitz. d. k. Akad. Wien*, LXVI. (iii), p. 102. 1872.

We found in one or two experiments that it was by no means easy to produce reflex slowing of the heart by any mode of excitation of the stomach wall. The most effective method was to paint a small area of the stomach wall with a weak solution of ammonia. In all modes of excitation the results obtained were the same. At first there is a slight rise in blood-pressure occurring with acceleration of the heart. After a variable period of about 10 seconds this is followed by a certain amount of slowing. These same results follow stimulations of the central end of the divided splanchnic, an observation which has been previously described by Roy and Adami[3]. It therefore seemed as if the whole of the afferent impulses passed up these nerves, but we found that division of the two vagi materially modified the reaction given when the stomach was again excited. In the one experiment in which only slight inhibition was being produced this was quite absent after the division of the vagi. In the second case in which the reaction was rather marked the slowing was distinctly less after the section. In both these experiments excitation of the central ends of the vagi produced only small effects upon the heart-rate, effects which were comparable to those obtained reflexly from the stomach. We therefore conclude that, in the cat, some of the afferent impulses which produce reflex alteration of the heart-rate are carried up the vagi as well as along the splanchnics.

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[1] Tarchanoff. *Arch. de Physiol.* VII. p. 498. 1875.

[2] François-Franck. *Travaux du Laboratoire de M. Marey*, II. p. 221. 1876.

[3] Roy and Adami. *Phil. Trans.* CLXXXIII. B. p. 258. 1892.

B. *The cardiac branches of the vagus.* To expose them the anterior halves of the upper three ribs were removed on both sides together with the corresponding portion of the sternum, the internal mammary arteries and veins being ligatured. The various cardiac branches were dissected out on both sides and threads passed under them. Each branch was stimulated, then cut and the central end excited with various strength of stimuli. The peripheral end was finally excited to determine if it contained inhibitory fibres. Sometimes this excitation failed to produce any inhibition though dissection proved that the branch in question entered the heart. We could not show that these particular branches had any greater inhibitory effects when excited centrally than others which contained many inhibitory fibres.

The results we have in this manner obtained may be tabulated in the following way.

(*a*) Slowing of the heart and a fall in blood-pressure.
(*b*) Slowing of the heart and a rise in blood-pressure.
(*c*) No alteration in rate of heart-beat but a fall in blood-pressure.
(*d*) No alteration in rate of heart-beat but a rise in blood-pressure.

We have not met any instances in which the rate of beat was accelerated. Excitation of the different branches in the same animal usually gave different results. Thus Figs. 2 and 3 give the results obtained by exciting the central ends of two cardiac branches of the right vagus in a dog. In Fig. 2 the highest cardiac branch of the right vagus was excited. It caused a fall in pressure and a slowing of the heart which persisted some little time after the excitation had ceased. In Fig. 3 the central end of the lowest branch (the sixth cardiac) of the same vagus was excited. It caused a decided rise in blood-pressure followed by a slight slowing of the rate, and with this slowing the blood-

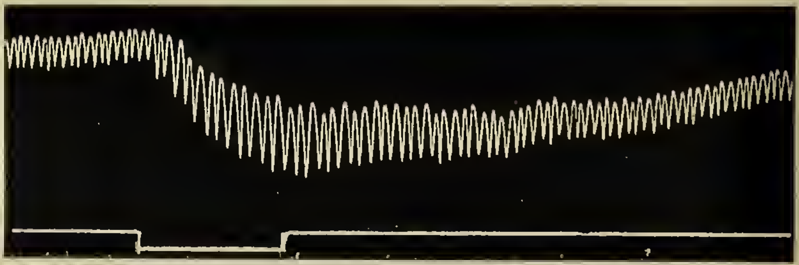

Fig. 2. Dog. A.C.E. Blood-pressure tracing. The zero-abscissa line has been raised 40 mm. At the time indicated on the abscissa line the central end of the uppermost cardiac branch of the R. vagus was excited. Coil at 5 cms.

pressure began to fall. Increase in the strength of stimuli applied did not produce a more marked effect with either of these two branches.

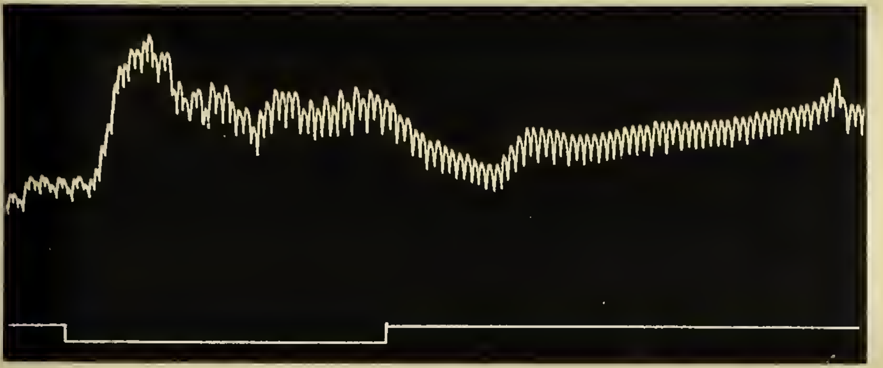

Fig. 3. Same dog as in Fig. 2. The zero-abscissa line has been raised 35 mm. At the time signalled the central end of the lowest cardiac branch of the R. vagus was excited. Coil at 5 cms.

The results of these experiments must be compared with those obtained by Wooldridge[1] by exciting the central ends of the various nerves which lie on the ventricular wall. By stimulating the central ends of the anterior ventricular nerves he found,

1. Slowing of the heart and a rise in blood-pressure.
2. Slowing of the heart and no effect on blood-pressure, and, as after effects, an increase in heart-rate and a fall in blood-pressure.

By exciting the central ends of the posterior ventricular nerves he found,

1. Increase in rate of beat and no alteration in blood-pressure.
2. Slowing of heart-rate and a fall in blood-pressure.
3. No effect on heart-rate and a slight rise in blood-pressure.
4. No effect on heart-rate and a fall in blood-pressure.

The results Wooldridge obtained by exciting the ventricular nerves thus entirely agree with the results we have obtained from the nerves after they have left the heart and before they join the vagi, with the exception of one class. These were the instances in which he observed accelerations of the beat following excitation of the posterior ventricular nerves. With the exception of a statement of Pawlow's[2], that excitation of a cardiac nerve in the thorax causes a rise in blood-pressure, we know of no other experiments dealing with stimulation of the central ends of these branches.

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[1] Wooldridge. *Archiv für Physiol.* 1883, p. 522.

[2] Pawlow. *Medicinisches Centralblatt*, 1883, p. 66.

In examining our results from the point of view of the strength of stimulus required to produce reflex inhibition, we show clearly that, as compared to the strength required when the central end of the vagus in the neck is excited, the stimulus must be distinctly greater. Moreover in the most marked cases the reflex inhibition is never very pronounced and there is never anything approaching to complete standstill.

C. *The pulmonary branches of the vagus.* These, as a rule, we only exposed upon the one side. The operation consisted in resection of the sixth, seventh, eighth and ninth ribs from close to the sternum to the transverse processes of the vertebræ. The branches were then easily isolated and tested in the manner described above when dealing with the vagi on the œsophagus. To our surprise we found that reflex inhibition can be obtained from any one of them with the greatest ease. Only a weak stimulus is requisite and the reaction is produced at once, results which stand in sharp contrast with these we obtained when working upon the other vagal branches. In these latter cases there is frequently a considerable latent period and the development of the reaction is gradual.

In Fig. 4 is reproduced a blood-pressure tracing showing the result of exciting one of these branches centrally. It is seen that the result is practically instantaneous and that the maximum amount of inhibition occurs during the early part of the stimulation. The result upon heart and blood-pressure is invariably the same and only differs in degree. The tracing we give may be regarded as an average result. In addition to the action upon the cardiac centre thus demonstrated, there is the usual action upon the respiratory centre and also one upon the vaso-

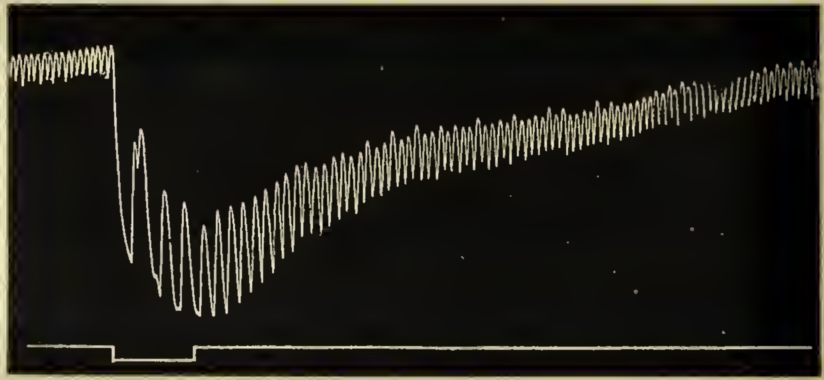

Fig. 4. Dog. A.C.E. Blood-pressure record. The zero-abscissa line has been raised 20 mm. At the time signalled on this line the central end of one of the pulmonary branches of the R. vagus was excited. Coil at 10 cms.

motor centre. This latter is demonstrable upon an animal in which the cardio-inhibitory fibres have been paralysed by atropine. Excitation of the pulmonary branches in such an animal causes a fall in blood-pressure which in some instances is very considerable. These results exactly agree with those one of us (T. G. B.)[1] has obtained upon the cat, as following the injections of a dose of serum.

The type of the reaction is as we have seen quite different from those produced on exciting the other branches of the vagus. It more nearly resembles the effect following stimulation of the peripheral end of the vagus divided in the neck.

The sensitiveness of the reaction is considerable. In most instances, it is easier to obtain reflex inhibition by exciting a single pulmonary nerve than by exciting the central end of the vagus divided in the neck.

This result is of further interest because it is exactly comparable to a result obtained by MacWilliam[2] in the eel. He found that slight stimulation of either gill causes a sudden and powerful inhibition of the heart's action, the whole organ standing still in a state of diastole. Stimulation of the central end of a branchial nerve, also led, with great readiness, to cardiac arrest. He notes that a similar inhibition can be produced in the carp, perch, rudd &c.

Our results with the pulmonary branches led us to examine the effect of various forms of stimuli upon the pleura. No effect could however be obtained although we tried electrical, mechanical, chemical and thermal forms of excitation.

While these experiments were in progress and soon after we had found the marked reflex effect upon the heart which follows stimulation of these pulmonary fibres a case came under the observation of one of us (A. E. R.), in which exploratory puncture of the lung resulted in the sudden death of the patient by cardiac failure. The case is of such interest in this connection that we give a brief account of it here.

The patient was a child in which the presence of pus in the lung or pleura was suspected. An exploratory puncture was therefore made using an ordinary hypodermic needle which was inserted for about a couple of inches. Only one puncture was made, no pus was found and the needle was withdrawn, the whole operation taking about thirty seconds. At the moment of withdrawal the child was observed to be livid in appearance, its hands were clenched and a convergent squint was present. The radial pulse could not be felt and no cardiac sounds could be heard with the stethoscope. Respiratory efforts however went on for a few minutes and were maintained for a short time by artificial means. In spite of this and other efforts, no further cardiac contraction could be detected. At the post-mortem examination no pus was present, the lung being in a condition of developing fibrosis following confluent broncho-pneumonia.

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[1] This number of the *Journal*, p. 48.

[2] MacWilliam. This *Journal*, VI. p. 233. 1885.

Having found the marked reaction following excitation of the pulmonary nerves we next examined the other afferent nerves supplying the remainder of the respiratory tract. Stimulation of the superior laryngeals produces quite as marked a reflex as that given by the pulmonary nerves, a result first described by François-Franck[1]. Stimulation of the recurrent laryngeals, as was to be expected, was quite ineffectual. Electrical stimulation of the nasal branch of the 5th nerve Kratschmer[2] found to be ineffective, a result which was however negatived by François-Franck. We are able to confirm the result obtained by the latter observer.

II. THE NATURE OF THE RELATIONSHIP OF THE RESPIRATORY TRACT TO THE CARDIO-INHIBITORY CENTRE.

The ease with which excitation of the pulmonary nerves is followed by cardiac inhibition at once recalls certain well known facts bearing upon this point. The first of these is the varying rate of beat observed in some animals during inspiration and expiration. During expiration the rate of the heart is slower, a difference at once abolished by dividing both vagi in the neck.

The difference in rate is usually well marked in dogs but is not as a rule to be observed in man, the cat, and the rabbit. To determine the cause of this modification in rate many experiments have already been recorded. The first worker in this direction was Einbrodt[3]. He found that if an animal be made to respire air under a positive pressure the heart was slowed. A similar result was observed if the air respired was under a negative pressure. Hering[4] showed that Einbrodt had missed one very important condition because the pressures he used were too excessive. If in the experiments in which a positive pressure was employed this pressure was kept low, *e.g.* 2 to 5 mm. of mercury, the positive ventilation causes acceleration of the heart. Einbrodt employed pressures of from 20 to 40 mm. of mercury. In a series of most instructive experiments Hering proves that the result is not due to any alterations in the mechanical conditions of the circulation affecting heart or vessels, nor to any alteration in the gaseous respiratory exchanges. He also proves that the acceleration during inspiration is due to an alteration of the tone of the cardio-inhibitory centre and that the afferent fibres which are excited are those of the lungs. Sommerbrodt[1] confirmed these results and moreover paid especial attention to the blood-pressure changes which accompanied them. He found that there was always a fall in blood-pressure, part of which was due to reflex dilatation of the blood vessels, and brings forward experiments to show that the rate of blood-flow through the capillaries is favoured by the fresh condition produced as a reply to the stimulation, which in itself tends to impede the flow. Léon Fredericq[2] however questions the interpretation of these experiments. He considers that the effect is not reflex but is an automatic action of the cardiac centre and is quite independent of any reflex activity that can be induced from stimuli originating in the lungs.

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[1] François-Franck. *Trav. du Labor. de Marey*, II. p. 221. 1876.

[2] Kratschmer. *Sitz. d. k. Akad. Wien.* LXII. (ii), p. 147. 1871.

[3] Einbrodt. *Ibid.* XL. (ii), p. 361. 1860.

[4] Hering. *Ibid.* LXIV. (ii), p. 333. 1871.

One of us[3] has described an effect produced by injecting serum into the circulation in a cat. This causes reflex arrest of the heart and of respiration and a diminution in tone of the vaso-motor centre. All these effects are cut out by section of the pulmonary nerves.

Before we were familiar with the details of the work of these observers we had independently carried out many experiments upon the same lines. We can entirely confirm the results arrived at by Hering. When a fairly high pressure is used for the respired air the rate of heart-beat may fall to one-half its former pace, but there is never that very marked slowing which follows excitation of the central end of one of the pulmonary nerves. We have also shown that during the whole of the time that artificial respiration is being carried out upon an animal the heart is constantly inhibited as a result of the inflation. This is true both in curarised and non-curarised animals. It is more particularly marked in animals in which the thorax is open, and in which the total volume changes of the lungs are consequently exaggerated.

With regard to the effect upon the vaso-motor centre we found, that suddenly changing the pressure of the respired air from atmospheric to a positive or negative pressure causes a fall in blood-pressure which is approximately proportional to the change in air pressure. This fall is also produced in atropinised animals and plethysmographic experiments show that it is then due to vaso-dilatation. The effect is cut out by dividing the pulmonary branches of the vagi. Hence it is a reflex inhibition of the vaso-constrictor centre in the medulla, just as the action on heart and respiration are produced in a similar manner. It is a very interesting fact that the mechanical excitation of these fibres produces exactly the same result as chemical excitation produced by injecting serum, in the experiments already referred to. The reaction to the mechanical stimulus is however never so pronounced as that given by a serum injection.

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[1] Sommerbrodt. *Zeitschr. f. klin. Med.* II. p. 601. 1881.

[2] Léon Fredericq. *Arch. de Biol.* III. p. 80. 1882.

[3] Brodie. This *Journal*, XXVI. p. 48.

We think it is important to note that, in these experiments in which the pulmonary nerves are mechanically excited, the fibres affected must be those in the alveolar walls.

The next experiments we wish to discuss are those in which variations in heart-rate accompany changes in blood-pressure. As was first pointed out by Marey, if the cardiac nerves are intact, a rise of blood-pressure is soon followed by slowing of the heart and conversely a fall in pressure produces acceleration. These alterations in heart-rate are reflex and it is generally considered that the afferent fibres which are excited and produce the effect arise from the heart itself. Considering the results we had obtained we thought it possible that the fibres stimulated in these experiments might be pulmonary fibres. We therefore tested the effect upon the heart-rate of a rise in pressure before and after section of the pulmonary nerves. The rise in pressure was produced in our experiments by injection of suprarenal extract, of nicotine, or by compressing the abdominal aorta. The effects were precisely the same after section of the pulmonary nerves as before. Hence we conclude that this reflex alteration in heart-rate is due to excitation of afferent cardiac fibres, for that it was not due to stimulation of any set of fibres below the pulmonary was proved by division of the vagi below these branches and subsequently injecting nicotine or compressing the aorta.

The last group of experiments we have to describe is one in which the pulmonary fibres were excited chemically by the inhalation of irritant vapours. We found this a most powerful method of producing reflex inhibition of the heart. Simultaneously, arrest of respiration always occurs whenever the vapour inhaled acts powerfully enough to cause inhibition. We obtained positive results by inhalation of the following vapours, viz.: chloroform, hydrochloric acid, formaldehyde and bromine. The latter was on the whole the most effective.

Before describing our results it will be best to refer to the somewhat similar experiments which have been described by Kratschmer[1] and François-Franck[2]. Kratschmer's experiments refer to excitation of the nasal mucous membrane. As irritant vapours he used cold air, carbonic acid, ammonia, acetic acid, chloroform and tobacco-smoke. He chiefly employed the last-mentioned because it gave a good reaction and was easily administered. He experimented chiefly on rabbits but confirmed his experiments upon cats. He was only able to obtain reflex arrest of the heart and respiration when the stimulus was applied to the nasal mucous membrane. When he excited the laryngeal mucous membrane by blowing in the gas from below and avoiding any possibility of it reaching the nasal mucous membrane by ligaturing the pharynx he only obtained negative results. He proved that the reaction was due to excitation of the nasal branch of the 5th nerve and that the olfactory nerve took no part in the production of the reflex. When any of the vapours were blown into the lungs, no result whatever was produced. In addition to the cardiac and respiratory effects produced in a positive reaction, the blood-pressure usually rose, or at any rate did not materially fall, from which he concludes that reflex vaso-constriction was also produced. François-Franck repeated these experiments but in some respects obtained different results. He entirely confirms the results of excitation of the nasal mucous membrane. He was however successful in obtaining reflex inhibition by the action of chloroform on the laryngeal mucous membrane. When he administered chloroform vapour by tracheal tube to the lungs and bronchi he however obtained negative results. He concluded that below the vocal cords the respiratory tract is insensitive to chemical stimuli.

We have repeated these experiments and can confirm those upon the nasal mucous membrane and upon the larynx. We have had no difficulty whatever in obtaining reflex cardiac inhibition by the action of chloroform vapour upon the larynx. This result stands well in conformity with the fact that reflex cardiac inhibition can be readily obtained by exciting the central end of the superior laryngeal nerve. With regard to excitation of the lower parts of the respiratory tract we cannot understand why François-Franck failed to get cardiac slowing by giving chloroform vapour, unless it is that he did not give enough of the vapour. We have frequently recorded slowing as an immediate result of chloroform administration. There is no question that this

[1] Kratschmer. *loc. cit.*

[2] François-Franck. *Trav. de Lab. de M. Marey*, II. p. 221. 1876.

part of the respiratory tract is less sensitive than the larynx and nasal mucous membrane. In fact as we proceed downwards along the respiratory tract the mucous membrane is found to be less and less sensitive to chemical irritants. If, however, a small drop of chloroform be placed on the trachea it is at once followed by marked cardiac inhibition. By making use of more irritant vapours, *e.g.* a small amount of bromine, the reaction on heart and respiration can be produced in an intense form.

In Fig. 5 we reproduce a curve showing how easily bromine vapour produces the cardiac inhibition when it is inhaled directly into the bronchi and lungs. The effect is, as we should expect, still more marked if the vapour can act upon the nasal mucous membrane or upon the larynx.

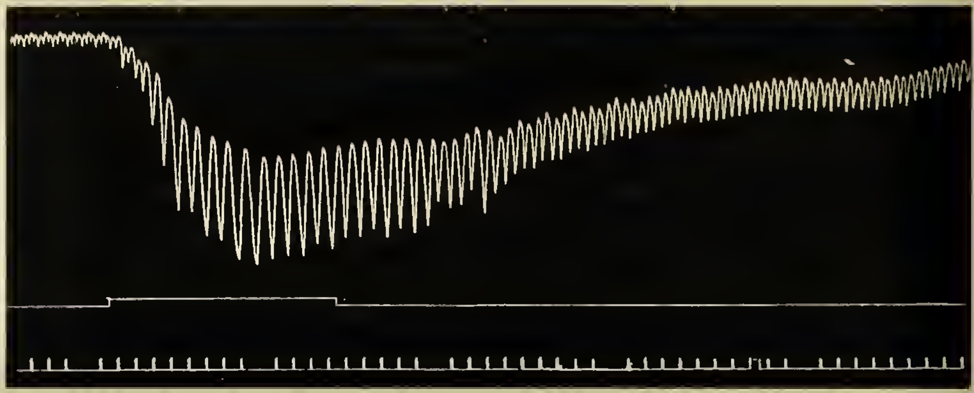

Fig. 5. Dog. Blood-pressure tracing. The zero-abscissa line has been raised 15 mm. Time tracing = seconds. During the time indicated on the abscissa line the animal was made to breathe out of a bottle containing a small quantity of bromine vapour. The animal was breathing through a tracheal cannula, so that the upper parts of the respiratory tract are not affected during the experiment.

The result produced in this experiment is practically identical with that following electrical excitation of the central end of a pulmonary nerve—the only difference being a less sudden onset, which may well be due simply to the fact that the vapour takes a little time to gain free access to the mucous membrane. The reaction is much quicker in onset and far more intense than that following mechanical excitation of the alveolar nerves.

That the reflex is, however, due to excitation of these pulmonary nerves is proved by Fig. 6, which was recorded at a later time from the same animal as in Fig. 5.

Between the taking of these two records, however, the pulmonary branches of both vagi were divided, and then another dose of bromine was given. The result is seen in the latter figure. The reaction is completely cut out. As a result of these and similar experiments we conclude that the alveolar nerves are the fibres excited, for only thus can we explain the fact that no reaction occurs in Fig. 6, although the fibres to the trachea are intact, and that the reaction, to bromine, is much more gradual in onset than that following stimulation of the pulmonary branches.

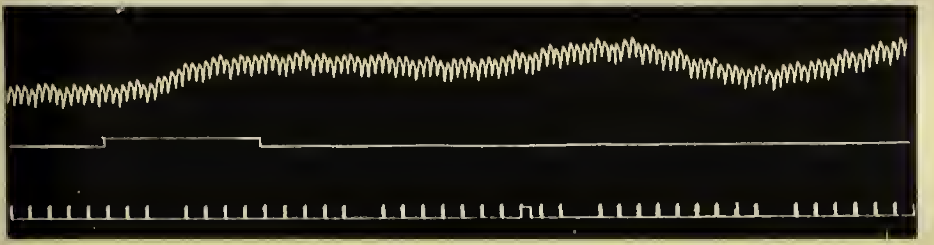

Fig. 6. Dog. Blood-pressure curve. The thorax has been opened and the pulmonary branches of the vagi have all been divided. At the time indicated upon the abscissa line bromine was again administered. It produced a rise in pressure and no slowing of the heart.

In attempting to determine whether the reflex has the same characters as those given by the excitation of the lung fibres, we gave the vapour to an animal previously treated with atropine. We still obtained arrest of respiration and a fall in blood-pressure, thus showing that the reflexes are exactly the same. In one experiment of this kind we observed a difference in the reaction, in that respiration instead of being arrested became quicker and shallower. Exactly this effect has also been observed following an injection of serum into a cat under atropine, so that here again we get confirmation of the precisely similar reactions obtained by the two different modes of chemically exciting these fibres. The correspondence between the two sets of experiments also confirms the view that the bromine in these experiments acts upon the alveolar nerves, for a study of the reaction given by serum leads conclusively to that result.

In attempting to form an idea why there is so close a relationship between the respiratory tract and these centres in the medulla we must note that the three chief effects produced by exciting the afferent fibres of the respiratory tract are inhibition of the heart, arrest of respiration, and vaso-dilatation. Further, Kratschmer has shown that excitation of the nasal mucous membrane leads to blocking of the respiratory tube by the bringing together of the vocal cords. In the next place the most sensitive surface is the nasal, *i.e.* the first surface of the tract, the one which would first meet any irritant entering the body by this path. The larynx follows this in sensitiveness, and then we have a long piece, the trachea and bronchi, which are apparently insensitive and lastly the alveoli which are again sensitive.

One of the conclusions which is obviously to be drawn is that the respiratory arrest at once stops further entrance of the irritant, and the circulatory disturbance tends to impede its absorption from the surface. Why the alveolar nerves should be in such close relationship to the cardiac and vaso-motor centres is by no means so clear, and we are not prepared to suggest any satisfactory explanation. The observation stands in unison with that made by MacWilliam and already referred to, namely, that stimulation of the gills in fishes leads to the same arrest of the heart.

CONCLUSIONS.

1. Of the branches of the vagus, excitation of the central end of which causes reflex inhibition of the heart, the pulmonary fibres are those which produce the most marked reaction. The cardiac are much less effective and the branches below the pulmonary are still less effective.

2. The connection of the respiratory tract with the cardio-inhibitory centre is very close. Thus: stimulation of the nasal mucous membrane at once arrests the heart. Stimulation of the laryngeal mucous membrane is only a little less effective. Stimulation of the trachea and large bronchi is apparently without effect, but stimulation of the alveolar nerves is about as effective as that of the laryngeal.

3. These nerves produce the result when excited electrically, in their course from the mucous membrane, or when stimulated mechanically or chemically in the mucous membrane itself.

4. In addition to the effect upon the cardiac centre, excitation of the pulmonary nerves also acts upon the respiratory and vaso-motor centres producing arrest of respiration and a fall in blood-pressure.

5. The alveolar nerves can be excited chemically by the inhalation through a tracheal tube of irritant vapours, such as chloroform, hydrochloric acid, ammonia, formaldehyde, or bromine, or by the injection of such substances as serum, or egg-white.

6. Division of the pulmonary nerves on both sides completely abolishes the effect upon the cardiac, respiratory and vaso-motor centres.

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THE IMMEDIATE ACTION OF AN INTRAVENOUS INJECTION OF BLOOD-SERUM.

By T. G. BRODIE, M.D., *Director of the Laboratories of the Royal College of Physicians, London, and the Royal College of Surgeons, England; Lecturer on Physiology, London School of Medicine for Women.* (Fifteen Figures in Text.)

(*From the Conjoint Laboratories of the Royal College of Physicians, London, and the Royal College of Surgeons, England.*)

In carrying out some experiments with antitoxic serum I was surprised to find that the intravenous injection of diluted blood-serum into a cat produced a marked effect upon the heart and respiration.

As serum is now being extensively used for therapeutic purposes I thought it would be well to follow this observation further. At the outset I must mention, that of the animals tested, dog, cat and rabbit, the effect is only to be observed in the cat. A second important point is that it is not a particular property of antitoxic serum for it can be obtained by injecting serum from any source, including even the serum obtained from the particular animal experimented upon. It was also found that several other substances, *e.g.* egg-white and the bromine compounds of proteids, could produce the same effect but in the experiments described in this paper serum has been chiefly employed. It may be mentioned that milk has a slight effect, but gelatine and peptone have none.

That serum is by no means an innocuous substance when injected subcutaneously or intravenously into an animal is already well known, but I need only refer to the most recent papers upon this subject. Weiss[1] describes a long series of experiments dealing with the toxicity of serum. The serum from all species of animal tested was invariably found to be toxic, though possessing different degrees of toxicity, and caused death if a sufficient quantity was injected. The symptoms produced as described by Weiss and other observers are briefly, diminution in the amount of urine and albuminuria, increased rapidity of the respiration and of the heart, and a rise in temperature. These symptoms might persist several days. If serum was continuously but slowly injected until it caused death, the respiration first became very rapid, then laboured, and finally the animal became convulsed. The rate of heart-beat was increased and at first the beat was stronger but gradually failed, though it persisted after the respiration had ceased. The pupils dilate and exophthalmos is produced. There is increased peristalsis of the small intestines. Death is due to paralysis of the respiratory and vaso-motor centres. Weiss chiefly employed rabbits for his experiments, but he obtained similar results with the cat and the dog. The degree of toxicity of different forms of serum for the rabbit has also been determined by Guinard and Dumarest[1]. They determine the quantities sufficient to kill within a few days. The most toxic serums are those of the ox, dog and cat; the least that of the horse. Another paper dealing with the same side of the subject is that of Friedenthal and Lewandowsky[2], which confirms and extends the previous work in this direction. They point out one further very important fact, viz., that if the serum be heated to 58°—60° C. for an hour it can then be injected without producing any symptoms of a toxic action[3].

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[1] Weiss. *Pflüger's Archiv,* LXV. p. 215. 1896. In this paper will be found full references to previous work. See also *Pflüger's Archiv,* LXVIII. p. 348. 1897.

In Fig. 1 is reproduced a typical tracing showing the effect produced by an injection of blood-serum upon the blood-pressure and respiratory tracings. The injection was made into the external jugular vein and it is seen that it is immediately followed by marked inhibition of the heart, the blood-pressure nearly falling to zero. Recovery of the heart sets in in about 10 seconds and then the blood-pressure begins to rise, at first rapidly as the rate of beat increases and then more slowly. After about 10 minutes the heart had regained its original rhythm and the blood-pressure had returned to its former height. The effect upon respiration is just as striking. Simultaneous with the arrest of the heart the respiration ceases in the expiratory phase, a prolonged and very slow inspiration follows and as the heart recovers a few irregular respirations occur, many of them being very deep, and then a second standstill occurs until 15 secs. later the normal rhythm once more returns. This is the ordinary effect produced by a fairly large injection

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[1] Guinard and Dumarest. *Comp. Rend. Soc. de Biol.* XLIX. pp. 414, 416, 495. 1897.

[2] Friedenthal and Lewandowsky. *Archiv für (Anat. u.) Physiol.* 1899, p. 531. See also Munk and Lewandowsky. *Ibid.* 1899, supp. p. 73.

[3] *l.c.* p. 539.

—in this instance 5 c.c. of normal horse serum—but several variations from this type are met with. The inhibition is commonly not so complete as in the case recorded, and recovery is then as a rule quicker. Moreover the effect on respiration is often different. Frequently the first change observed is the occurrence of two or three shallow and rapid respirations followed by arrest in expiration, and usually the tracing at this time is quite horizontal. On a few occasions I have observed as the first effect the occurrence of two or three deeper and slower respirations before the arrest.

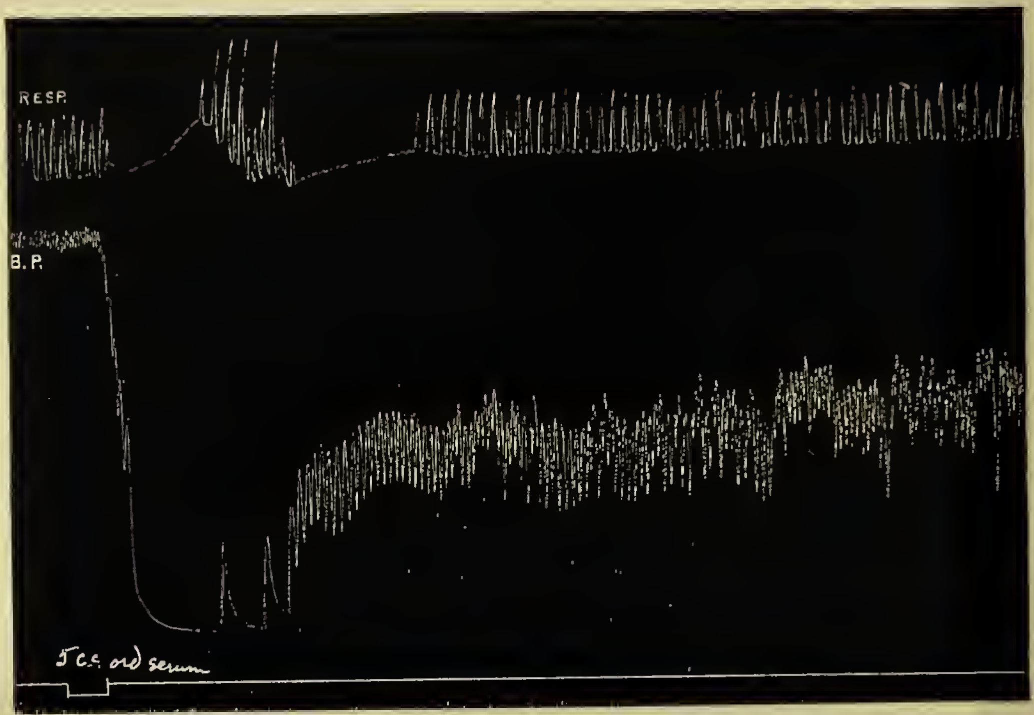

Fig. 1. Cat under urethane[1]. The upper tracing is that of respiration. Rise of the lever indicates inspiration. The second tracing is that of the blood-pressure by a mercury manometer connected to the carotid. The lower line is the abscissa line of zero-pressure. At the time indicated by a break in this abscissa line 5 c.c. of normal horse-serum was injected.

In other instances the respiration simply becomes shallower (Fig. 11) and this is followed by quicker respirations which are also frequently deeper.

The recovery of respiration is, as a rule, not so sudden as in Fig. 1, but on returning the rate and depth gradually increase and may for a time exceed the normal. (See Fig. 15.) Occasionally animals have been met with which show no effect whatever upon the respiration although the cardiac effect is typical.

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[1] All the figures in this paper are to be read from left to right.

In addition to the two well-marked results already described a third effect, viz. one upon the vaso-motor centre, is also produced. The experiments proving this will be described later.

One of the most striking features of the reaction is the great suddenness of the changes in the heart and respiration and the fact that both are identical in point of time.

My experiments upon this result of a serum injection have been chiefly concerned with four main points, and it will therefore be most convenient to describe them in four sections. In the first of these the mode of production of the arrest of the heart and respiration is discussed; in the second the effect upon the vaso-motor system; in the third the production of immunity to a serum injection, and in the fourth the constituent of serum which produces the reaction.

Throughout the experiments the animals were anæsthetised with the ordinary A.C.E. mixture, though in a few instances urethane was employed.

The amount of serum required to produce the result varies considerably with the animal, often as little as 0·1 c.c. produced a typical reaction, whilst at times as much as 10 c.c. had to be injected. On a few occasions the animals produced no reaction at all. The time of year has also seemed to have some influence, for the animals appeared to be more sensitive in the winter months than in the summer.

As a rule the injections have all been made into the jugular vein, but the result is exactly the same when the injection is made into the femoral vein or into a branch of the mesenteric vein.

I. THE MODE OF PRODUCTION OF THE ARREST OF HEART AND RESPIRATION.

The fact that the action on the heart and respiration occurred simultaneously seemed to indicate that the action was one on the medulla, but this is at once negatived by cutting both vagi in the neck after which a serum injection produces very little effect. This is seen in Fig. 2 taken from an animal which had given a well-marked reaction to an injection of 3 c.c. of cat's serum before the vagi were cut in the neck. In the experiment given as much as 10 c.c. were now injected and the only result observable is a small and transient fall in blood-pressure. There is no slowing of the heart and practically no alteration of respiration. The cause of this fall in blood-pressure will be discussed later. The conclusion to be drawn from these two results

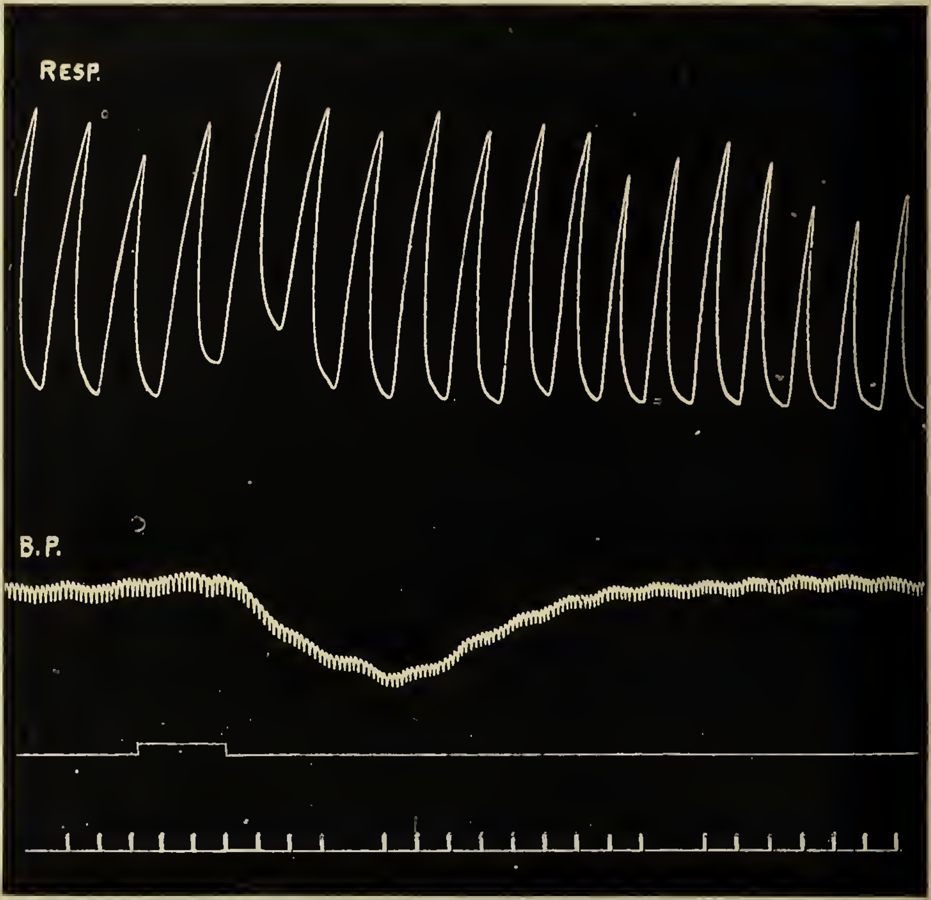

Fig. 2. Cat. Respiration and blood-pressure. The vagi have been previously divided in the neck. The zero-abscissa line has been raised 55 mm. At the time signalled on this line 10 c.c. of cat's defibrinated blood was injected. Time record = seconds.

is that some substance in the serum excites the terminals of some set of afferent fibres of the vagus, and these impulses act upon the medullary centres and produce, as their chief effect, slowing of the heart and arrest of respiration.

The stimulation of these fibres lasts a considerable time after the serum has been injected. This is well seen from Fig. 3, in which experiment the vagi were cut in the neck during the effect upon the heart and respiration. Immediate recovery follows. In one experiment the section of the vagi led to failure of the heart and death of the animal. I have repeated this experiment many times but have never, with this single exception, obtained any result differing from that recorded in Fig. 3.

In some cases the excitation of the vagal fibres lasts so long as to cause the death of the animal. This I have often observed with doses as large as 10 c.c. and on one occasion with 2 c.c. of serum.

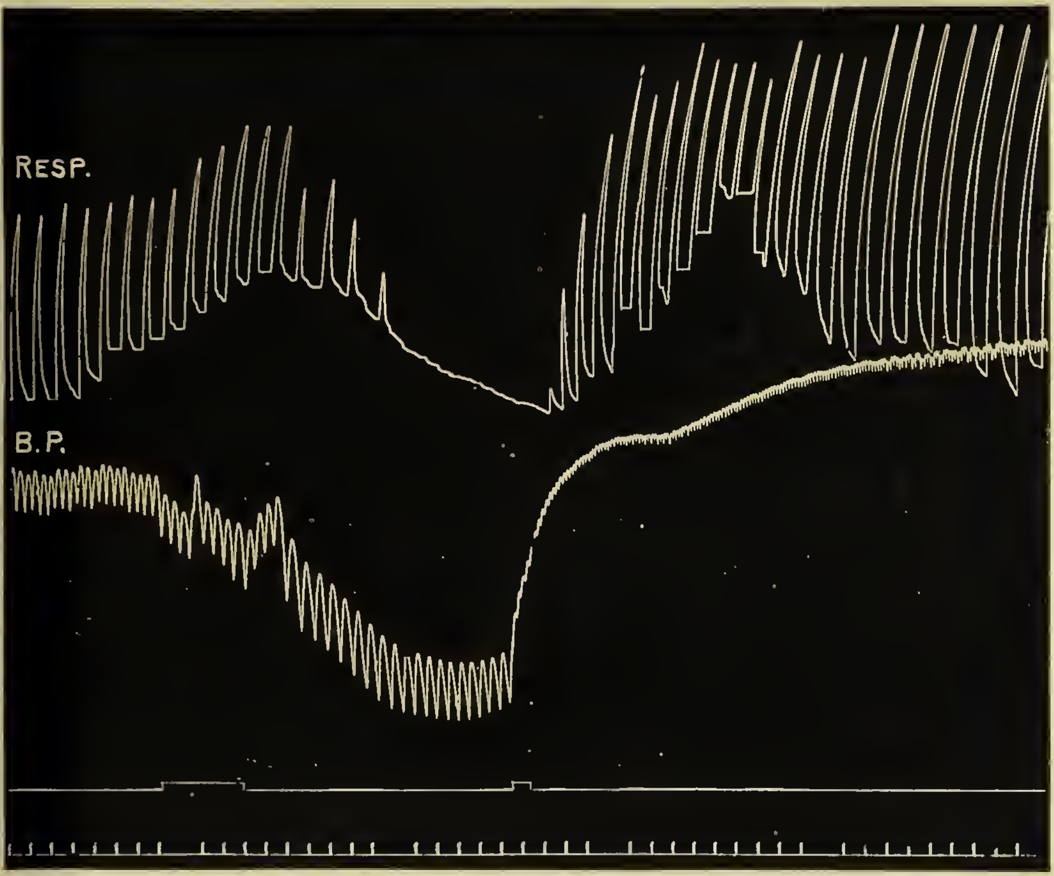

Fig. 3. Cat. Respiration and blood-pressure. Zero-abscissa line has been raised 15 mm. At the first signal on this line 10 c.c. cat's defibrinated blood was injected. At the second the two vagi were divided in the neck. Time record = seconds.

That the action of the serum is upon the periphery and not on the medulla is further shown by injecting the serum into the peripheral end of the carotid artery, when the reaction should occur more quickly and in greater intensity if the action were one upon the medulla. This is, however, not the result, but on the contrary there is a considerable latent period before the typical reaction occurs. During this latent period no alteration in the blood-pressure or respiratory rhythm was, as a rule, observed. In other instances, the injection was immediately followed by a rise of blood-pressure which suddenly fell later as the usual reaction was produced. In these injections into the carotid less effect is, as a rule, observed upon the respiratory rhythm.

It being evident that the effect is a reflex one the afferent impulses being carried along the vagus, I performed several experiments to determine which were the vagal fibres stimulated. The general plan of these experiments was to first insert cannulæ in one carotid, one external jugular and the trachea and then to expose the vagus in the chest. In the first instance the two vagi were obtained on the œsophagus. For this purpose the thorax was opened by resection of three ribs, artificial respiration being established. The two vagi were isolated and loops of thread passed round them. The carotid was then connected to the manometer and a dose of serum injected. The vagi were next gently lifted up by the threads and divided. This was followed by a second injection of serum.

In other experiments the pulmonary branches and the cardiac branches were similarly treated. These fibres were obtained by removing the upper half of the sternum and the anterior portions of the upper three or four ribs. The fibres were then isolated and the experiment completed as above.

In another set of experiments the left vagus was cut in the neck and then the various branches of the right vagus were isolated and the action of serum tested before and after their section. Both methods yielded similar results, which were as follows:

(i) Section of the vagi on the œsophagus below the root of the lung does not in any way modify the result. In a few instances the effect has not been so well marked as in the control injection, but this is due to a cause explained later (see pp. 56 et seq.). Hence we can conclude that the afferent fibres, stimulation of which produces the reaction, are not contained in any of the vagal branches below the pulmonary.

(ii) Section of all the cardiac branches of both vagi only produces a modification of the reaction due to the absence of the reflex cardiac effect (Fig. 4). The result obtained here is directly comparable to that seen in an atropinised animal (Fig. 12). There is still in both cases a marked fall in pressure. As artificial respiration had to be employed in the experiments when the nerves were divided it was very difficult to determine any alterations in the respiratory rhythm. This difficulty was overcome in the following manner. When everything was ready for the serum injection the amount of artificial respiration was gradually decreased until the animal made voluntary respiratory movements and the serum was then injected. The movements at once ceased. The cardiac branches were next cut and serum a second time injected under similar conditions. Again the respiratory movements ceased. Hence it follows that the cardiac fibres of the vagus do not contain any afferent fibres, stimulation of which by serum can cause the arrest of respiration or depression of the vaso-motor centre. Moreover, from the results recorded in the next paragraph it is certain that the reflex arrest of the heart is not due to excitation of any afferent fibres in the cardiac branches of the vagus. In all cases care was taken that all the cardiac branches were cut. This was tested by stimulating the two vagi in the neck after the division of the branches in question and observing that no inhibition resulted.

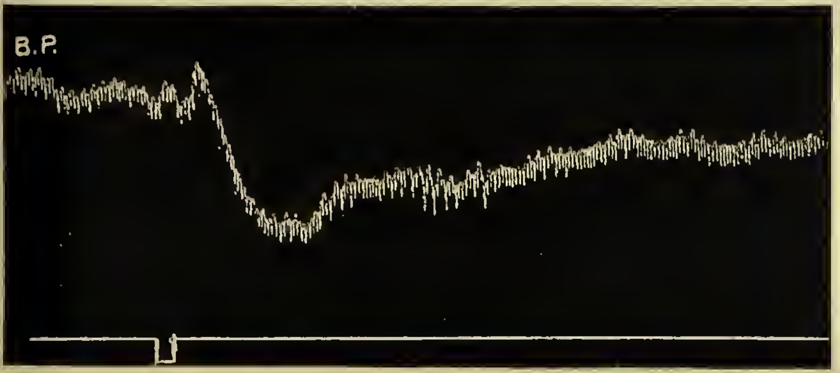

Fig. 4. Cat. Thorax opened. Blood-pressure tracing. The zero-abscissa line raised 30 mm. The cardiac branches have been previously divided in the thorax. At the time signalled 3·5 c.c. horse-serum was injected.

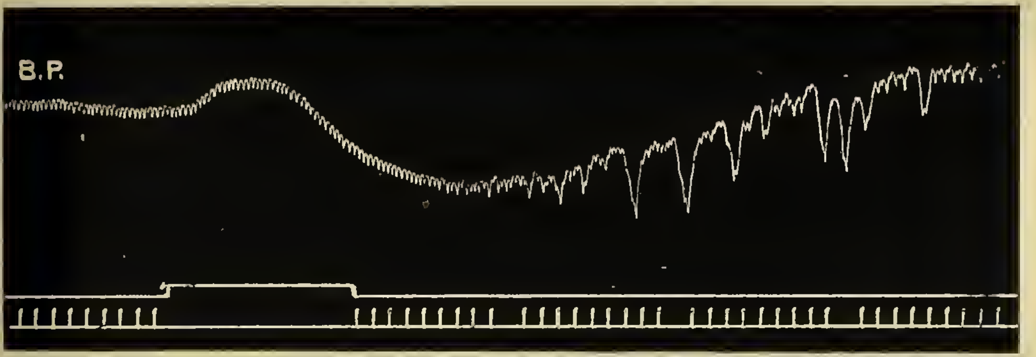

Fig. 5. Cat. Thorax opened. Blood-pressure tracing. The zero-abscissa line has been raised 40 mm. The pulmonary branches of the vagi previously divided where they enter the root of the lung. Result of injecting 10 c.c. horse-serum. Time record = seconds.

(iii) Section of the pulmonary branches completely cuts out the reaction (Fig. 5). The behaviour of the blood-pressure curve is exactly the same as after the division of the vagi in the neck. (Compare Fig. 5

with Fig. 2.) A subsequent injection after the vagi have been divided in the neck shows no further modification. The action on the respiratory rhythm was tested as described under (ii) p. 54, and the respiratory reflex also found to be abolished.

Hence it is the pulmonary afferent fibres stimulation of which by serum produces in the cat reflex arrest of the heart and respiration.

This result is of further interest when taken in conjunction with the results obtained by Russell and myself[1] upon reflex cardiac inhibition.

During the course of the experiments in which the thorax was opened it frequently happened that the reaction to a serum injection failed. This often occurred even with animals in which a typical reaction had been obtained before the major part of the operation had been commenced, thus proving that it was not due to any idiosyncrasy on the part of the animal. I at first thought it might be due to the production of some degree of immunity due to the absorption of some of the serum from the blood escaping during the operation. Great care was therefore taken to prevent all bleeding during the operation but still the same results were obtained. For a long time this proved a great hindrance to the completion of the experiments and the cause and the way in which it was to be avoided was only discovered when the work was nearly completed. After I had found that the whole reaction was due to the excitation of the pulmonary nerves and after Russell and I had shown how readily cardiac inhibition could be produced by electrical stimulation of these same branches it struck me that the failures just mentioned might be due to the artificial inflation of the lungs. I argued that the forcible inflation might be exciting these fibres so much that they became insensitive to the serum injection. In favour of this idea, we know that if in an animal, with the thorax intact, artificial respiration is established the animal soon ceases to make voluntary respiratory efforts unless the degree of inflation is slight. In my experiments the force of the air-pump used for the lung inflation was kept low and the lungs only distended so far as to nearly fill the thorax. Still the repeated distension of the lungs might be producing so much mechanical excitation of the pulmonary fibres that they became insensitive to the serum. On the other hand the pulmonary fibres might be carrying up such strong impulses to the bulbar centres that the impulses started by the serum could produce no effect. For the purpose of testing these views the following experiments were performed.

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[1] See this *Journal, infra.*

The chest was opened as if for an operation upon the cardiac nerves, artificial respiration being carried out. Then, while the blood-pressure was being recorded the extent of inflation of the lung was considerably decreased. When this had been carried out for some time and the rate of heart-beat had become steady the amount of air pumped in at each distension of the lung was suddenly increased. This caused considerable inhibition. The amount of air was next decreased, when the rate of beat gradually quickened. Complete and sudden cessation of artificial respiration caused as a primary result acceleration of the heart. These results upon the heart at once showed that its rate could be materially modified by impulses started from the pulmonary fibres of the vagus. Hence as both respiratory and cardiac rhythm can be modified by the same excitation of the pulmonary nerves, it seemed likely that that excitation would materially modify the action of serum when injected, since as we have just seen it acts upon the same nerve terminals. The following experiments were therefore tried. In the first the animal was prepared as for an ordinary injection and a cannula tied in the trachea. A dose of serum was now injected to show that the animal gave a typical reaction. The tracheal cannula was now connected to the respiration pump and, the thorax still being intact,

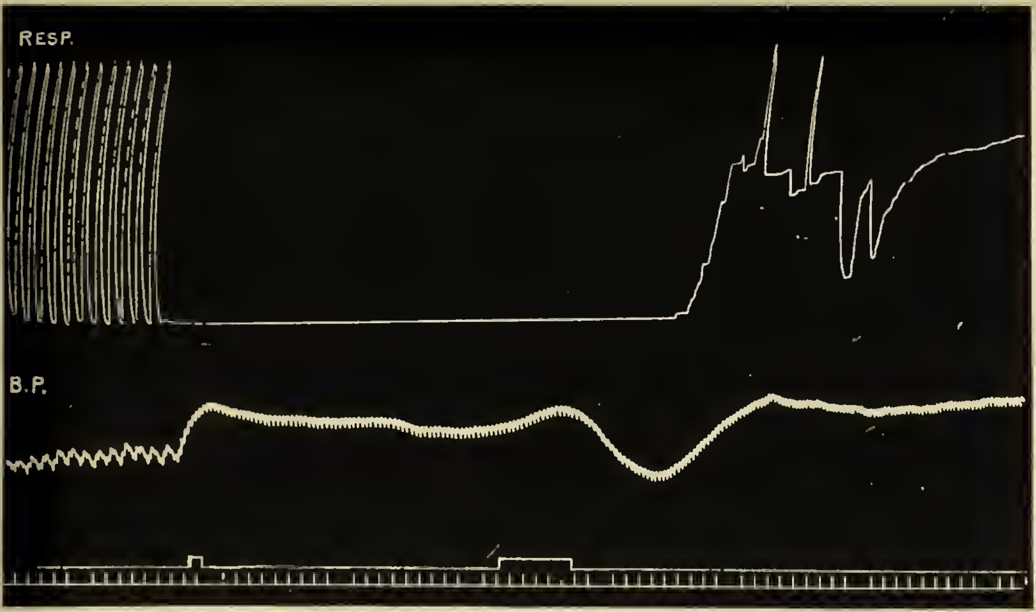

Fig. 6. Cat. Thorax intact. Upper tracing, respiration. Lower = carotid blood-pressure. Zero-abscissa line raised 40 mm. The thorax is being artificially inflated up to the first signal. At the second and during the apnœa 4 c.c. horse-serum was injected. Time record = seconds.

artificial respiration was started, using a degree of distension a little greater than normal. The animal's respiratory movements soon ceased and the heart beat somewhat slower. After a time the artificial respiration was stopped and, during the period of apnœa which followed, a dose of serum injected. It was found to give only a slight fall in blood-pressure and no inhibition of the heart. The effect upon respiration was to produce inspiratory tetanus (Fig. 6). In another experiment the mechanical excitation of the pulmonary fibres was brought about by partially blocking the tracheal cannula, so as to cause marked dyspnœa. The reaction was markedly altered (Fig. 7). Respiration was a little slowed, the rate of the heart was not altered, and there was only a moderate fall in blood-pressure.

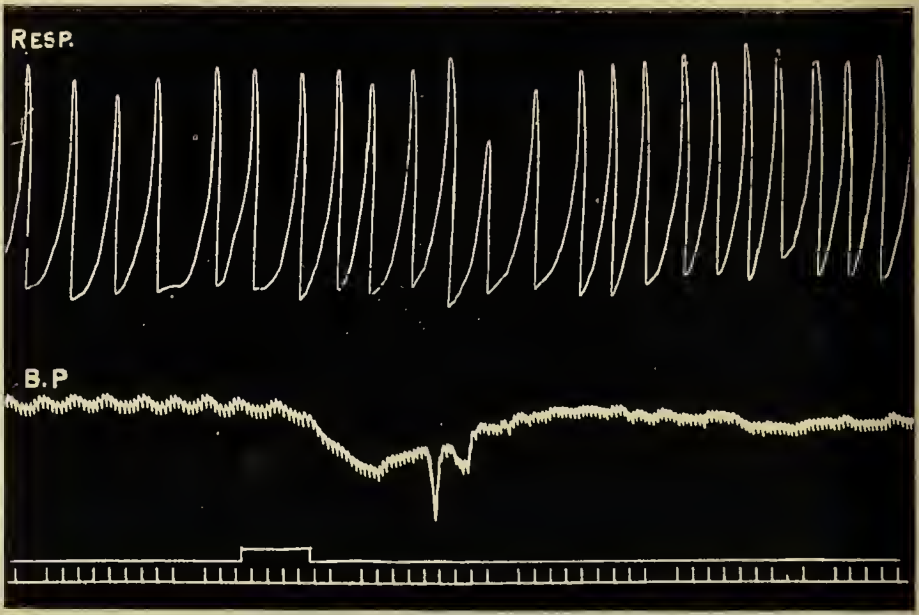

Fig. 7. Cat. Thorax intact. Respiration and blood-pressure. Zero-abscissa line has been raised 35 mm. Cannula tied in trachea. This is partially blocked during the record. At the time signalled 4 c.c. horse-serum was injected.

Similar experiments were also performed upon an animal in which the thorax was opened by the removal of the first rib on either side. A dose of serum injected while artificial respiration was being continued produced only a slight fall in blood-pressure and no slowing of the heart (Fig. 8). In the next place the artificial respiration was diminished in extent until spontaneous respiratory movements appeared. The artificial respiration was then completely stopped, and 30 seconds later serum injected (Fig. 9). The effect produced was a good fall in blood-pressure and marked slowing of the heart. The effect on respiration was first to accelerate and shorten expiration, and then to produce a tendency towards expiratory standstill. Artificial respiration then had to be restarted when gradual recovery took place.

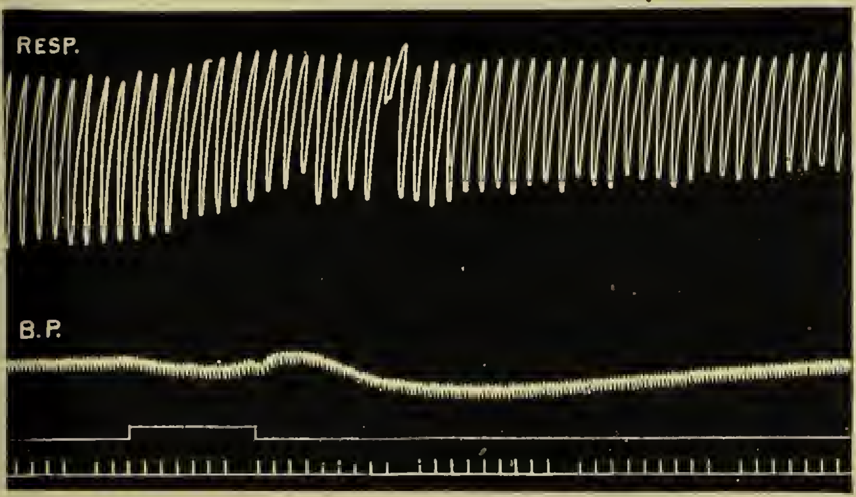

Fig. 8. Cat. Thorax opened at upper end. Respiration and blood-pressure. Zero-abscissa line has been raised 50 mm. The force of inflation of the lungs was only slight and carried out so as to cause the lungs to nearly fill the thorax with each inflation. At the time signalled 5 c.c. horse-serum was injected.

These experiments also explained the result which I had usually obtained upon curarised animals. Here again a similar result is produced if the serum be injected while the artificial inflation of the lungs is continued. There is only a fall in blood-pressure, but no effect upon the rate of beat of the heart. If on the other hand the respiration is stopped, and serum injected early in the course of the asphyxia which follows, a quite typical reaction upon the heart is recorded.

II. THE ACTION OF SERUM UPON THE VASO-MOTOR SYSTEM.

In addition to the effect the afferent vagal impulses produce upon the cardiac and respiratory centres, they also act upon the vaso-motor centre inhibiting its tonic action. A serum injection also acts upon the blood vessels apart from this action through the bulbar vaso-motor centre, as is seen for instance in the tracings obtained after both vagi have been divided in the neck.

That there is a reflex action upon the blood vessels through the bulbar vaso-motor centre follows from a study of the following points. In the first place the great fall in blood-pressure produced by an

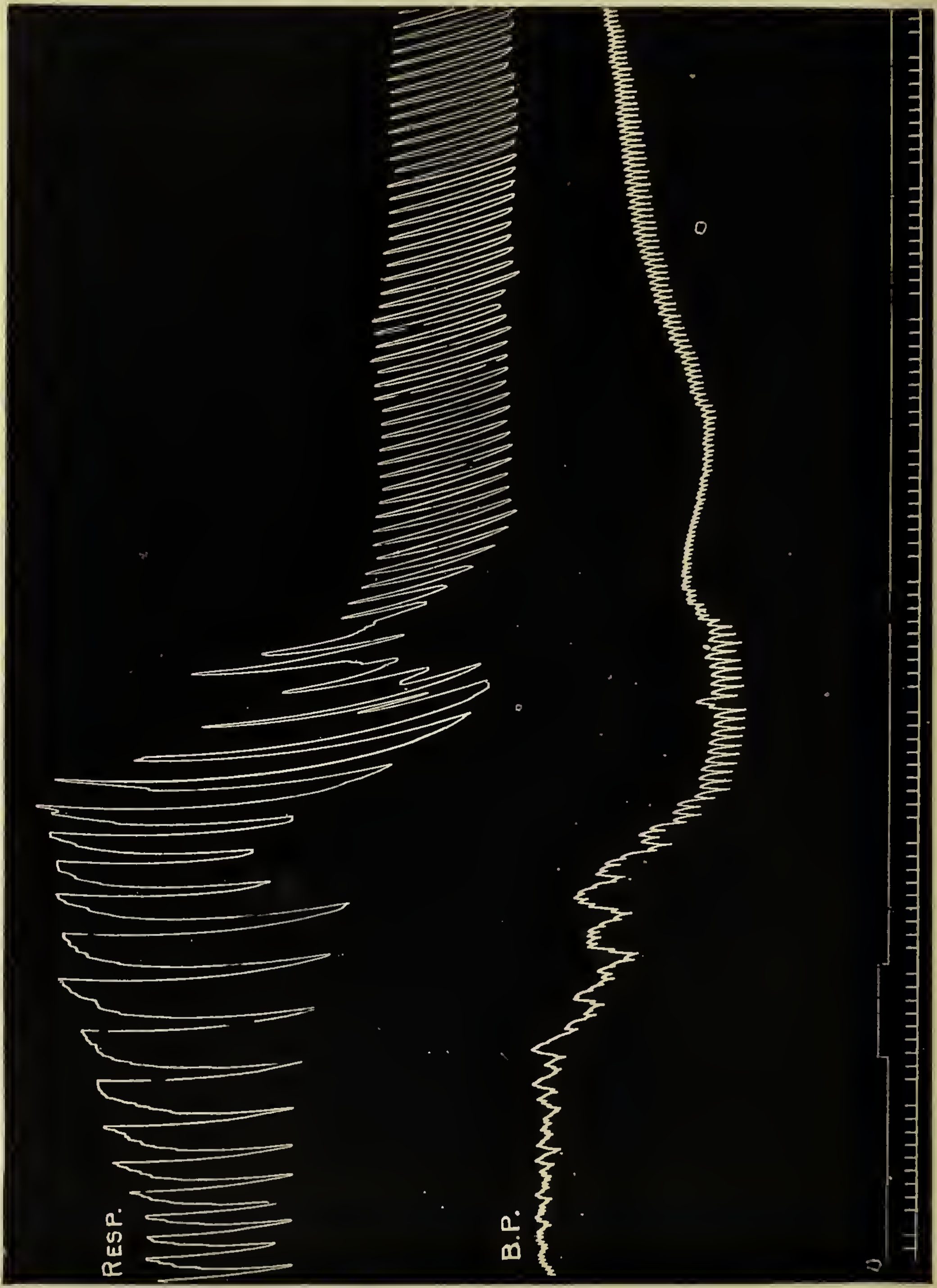

Fig. 9. Cat. Same animal as in Fig. 8. The artificial respiration was stopped 20 secs. before the commencement of the tracing and the animal was making respiratory efforts as indicated by the upper record which gives the abdominal movements. The thorax open. At the first signal 5 c.c. horse-serum was injected. At the second artificial respiration restarted.

injection cannot always be explained by the amount of cardiac inhibition produced. In some animals only slight, and in others no slowing of the heart occurs (Fig. 10), although the blood-pressure falls considerably, a result which is commonly seen in animals into which several injections of serum have been previously made. In the next place a marked fall in pressure follows an injection made after all the cardiac branches of both vagi have been divided, thus eliminating the effect due to inhibition (Fig. 4).

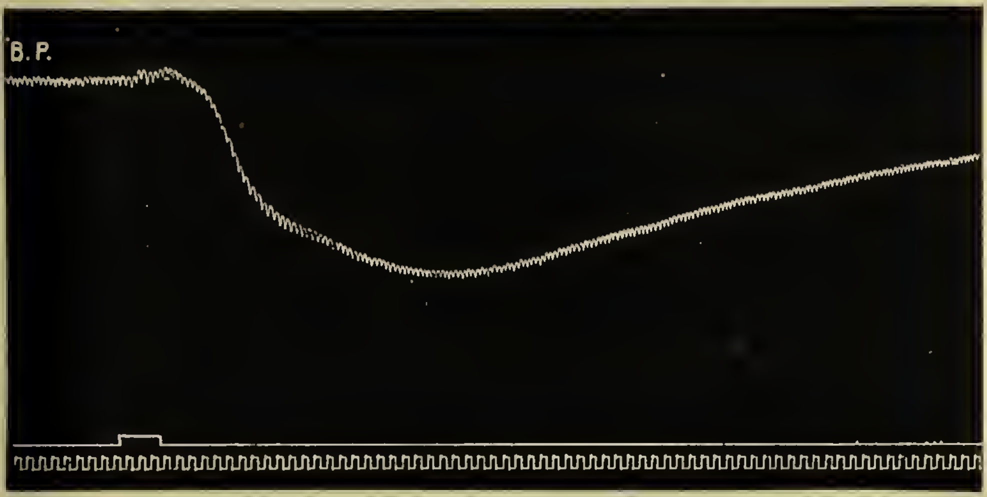

Fig. 10. Cat. Blood-pressure. Injection of 10 c.c. horse-serum. Time-tracing = seconds.

Again, in animals in which inhibition has been prevented by a preliminary injection of a small dose of atropine, serum still produces a marked fall in pressure (Fig. 12). The fall in pressure in these two instances might be due to a direct action of the serum upon the heart muscle. This influence is however practically negligeable, for oncometric records of the heart-beat show but very slight changes following an injection. Moreover, in either of the two cases just mentioned, in which cardiac inhibition is avoided, if the vagi be divided in the neck, a serum injection now produces a much smaller fall in pressure than that occurring before the division of the nerves.

I have also studied the action upon the blood vessels directly by means of the plethysmographic method. The intestinal plethysmograph shows a dilatation occurring with the fall in blood-pressure (Fig. 11). The fall of pressure, some of which is of cardiac origin, would in itself cause a diminution in volume of the intestine, but the dilatation of the vessels is sufficient to more than counterbalance the effect due to the heart.

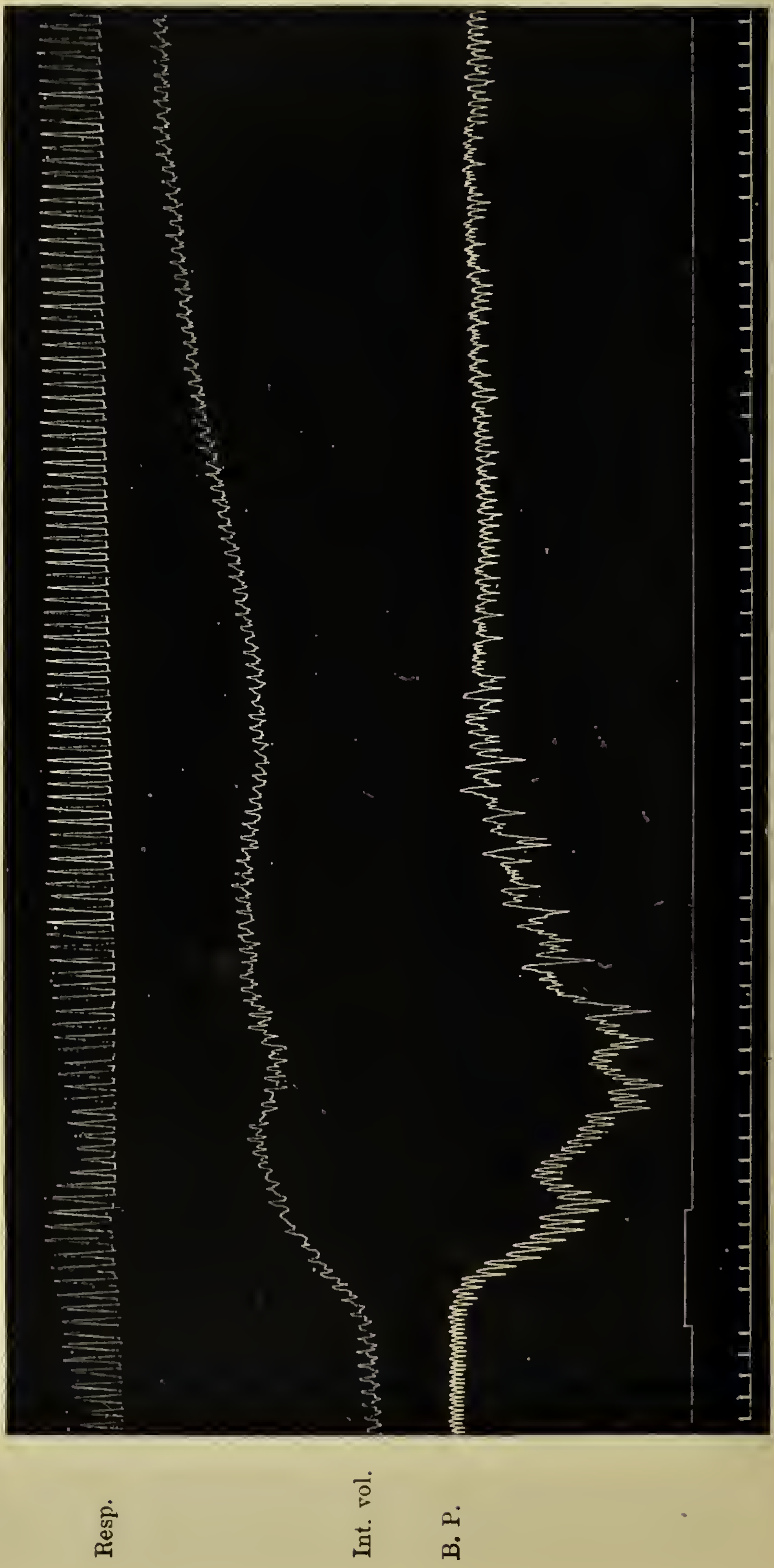

Fig. 11. Cat. Upper tracing = respiration. Second = volume changes of small intestine. Lower = blood-pressure. Injection of 5 c.c. dog's-serum. Time-tracing = seconds.

After the heart has regained its initial rate, the intestinal vessels still show a very decided dilatation. Plethysmographic records of the heart prove that at this stage the output of the heart is quite at its original value. In a few experiments I have found that the intestinal tracings record no alteration in volume, *i.e.* the volume decrease due to the fall in pressure is exactly counterbalanced by the increase due to vaso-dilatation.

If the record be taken upon an atropinised animal, the action on the blood vessels stands out still more clearly. Thus Fig. 11 shows a very marked dilatation of the intestinal vessels which commences a little earlier than the fall in blood-pressure. This tracing is of further interest, for it records an effect upon respiration which is to be frequently observed in atropinised animals. Instead of the respiration becoming arrested, it is quickened and becomes a little shallower. To obtain arrest a much larger dose of serum must be injected.

That most of the vaso-dilatation is due to an action upon the vaso-motor centre follows from the record of the effect produced by a larger dose of serum after both vagi had been divided in the neck.

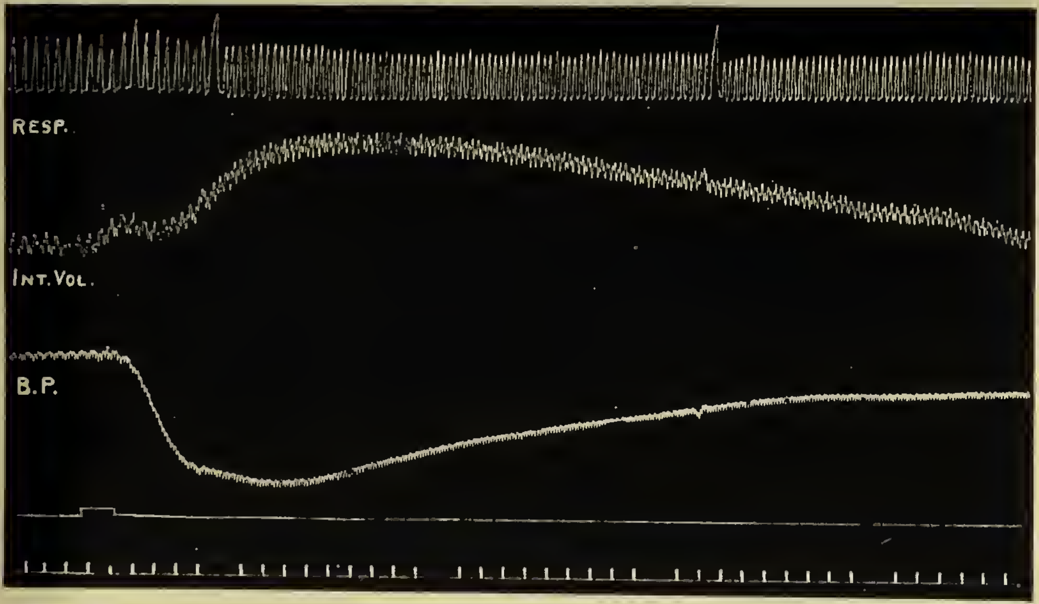

Fig. 12. Same animal as in Fig. 11, but 1·5 c.c. of a 0·5 % solution of atropine has now been injected. At the signal 5 c.c. of dog's-serum was injected.

Most of the effect upon the intestine is in this way abolished, but there is still some fall in blood-pressure. The fall in pressure rarely exceeds that seen in Fig. 2, p. 52, and the effect upon the intestinal plethysmograph is in proportion.

From plethysmographic records, taken after division of the vagi in the neck, one can also show that the fall in pressure which now occurs is almost entirely due to vaso-dilatation. This is the case in atropinised animals as well as in those which have not received a dose of the drug. I have been able to exclude the possibility of the effect being due to a direct action of the serum upon the heart by recording the volume changes of the heart during an injection. The volume remains nearly unaltered, only a slight diminution in output per beat, and no alteration in rate taking place.

As to how the serum acts when both vagi are divided, I have not been able to decide. It is not a direct action of the serum upon the vaso-motor centre in the medulla, for an injection into the carotid does not increase the effect, it only delays its onset.

In studying the behaviour of the vaso-motor system after an injection of serum, other organs, *e.g.* the limbs, the lung, the testis, have been examined plethysmographically but they all, with one exception, only confirm the results that have been described above. This exception is the kidney, which behaves in a totally different way. Instead of dilating as a result of serum injection the kidney diminishes in volume. This decrease in volume is due to an active constriction of the kidney vessels, for if the tracings are examined it is seen that the waves due to the heart-beat are considerably diminished in extent and in most cases completely obliterated. Moreover, as a rule, the decrease in volume does not correspond to the variation in blood-pressure, for the constriction usually persists much longer than the fall in pressure, in some instances it continues to increase more than a minute after the blood-pressure has quite recovered. This prolonged constriction is followed by a slow relaxation which may extend beyond that recorded before the injection was made. If the kidney is watched during these changes the constriction of its vessels is easily followed, for marked pallor is produced. The result of an injection into an atropinised animal also shows this change well (Fig. 13). From this tracing it will be seen that the volume changes do not coincide with the fall and rise of blood-pressure though in this particular instance this is not so marked as is frequently the case. The heart-beats on the kidney tracing are also seen to greatly diminish with the decrease in volume. Later in the same experiment an injection, after both vagi had been cut in the neck, caused only a slight fall in blood-pressure and the kidney volume scarcely changed. The result is not always so marked but all lead to the conclusion that the constriction of the kidney is a reflex and is due to an excitation of the vaso-motor centre in the medulla.

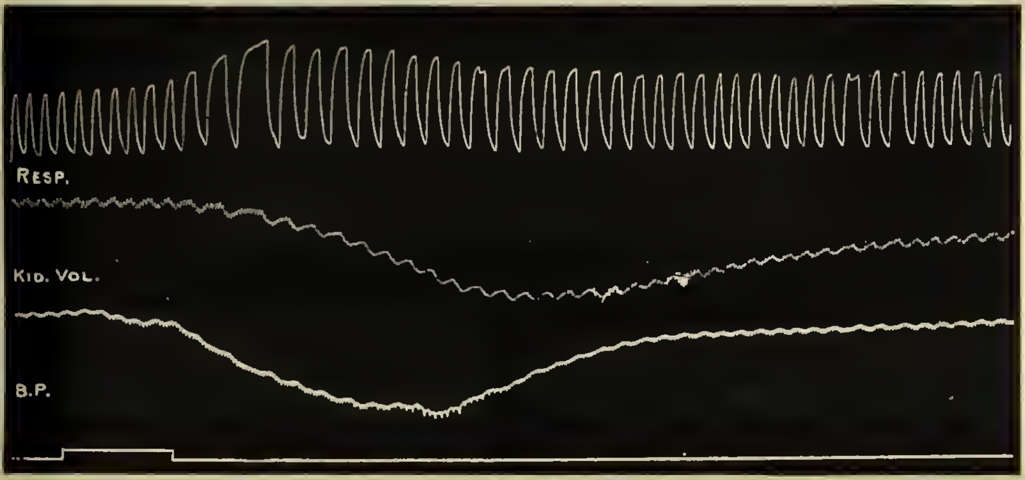

Fig. 13. Cat. Respiration. Volume changes of R. kidney. Blood-pressure. The animal has been previously injected with 2·0 c.c. of a 0·5 % solution of atropine. Injection of 5 c.c. horse-serum.

That the kidney vessels should behave in the opposite sense to the intestinal vessels is a fact of considerable importance. It has been observed among others by Halliburton and Mott[1] for cholin and neurin and by Dixon[2] for spermine, though it was not shown to be due to an action on the medullary centre. The result I have obtained modifies the general conclusion drawn by Porter and Beyer[3] as to the action of the bulbar vaso-motor centre. They consider that all afferent impulses which act upon the bulbar vaso-motor cells influence them all alike and that this centre therefore plays no part in the distribution of the blood to the several organs and regions of the body. My experiments indicate that this is not always true and that the kidney may prove an exception. Further experiments upon this point are now in progress.

III. Effect of repeated injections. Production of a condition of immunity.

Early in the course of these experiments I found that, if several injections were made into the same animal, the later injections produced a much less marked effect than the first. The more frequent

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[1] *Phil. Trans.* B. cxci. p. 211. 1899.

[2] This *Journal*, xxv. p. 356. 1900.

[3] *Amer. Journal of Physiol.* iv. p. 283. 1900.

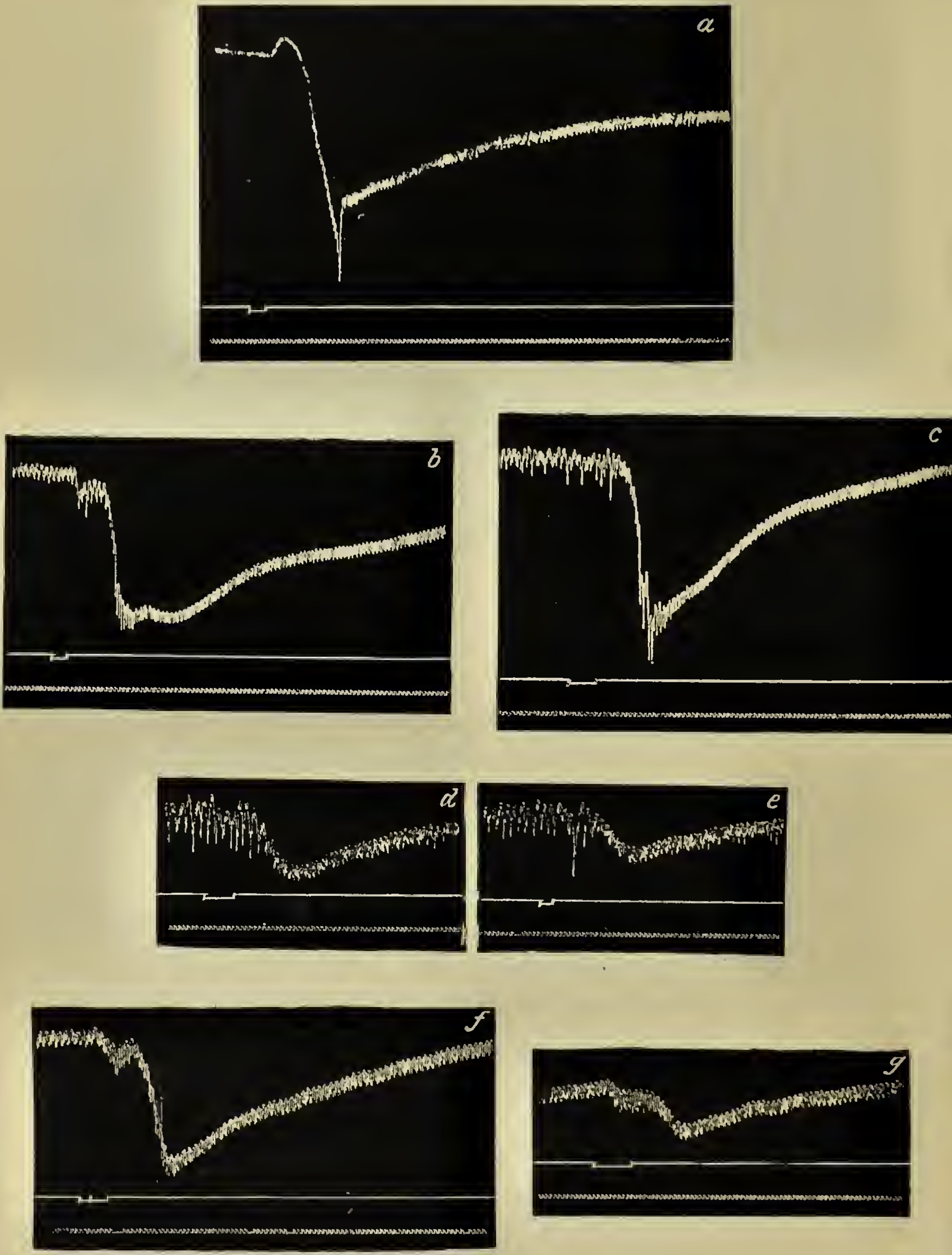

Fig. 14. Cat. Blood-pressure records. A series of successive injections of 5 c.c. horse-serum. In each case the blood-pressure started at approximately the same level. The first injection at 3 hrs. 0 min. p.m. Tracing (*a*). The respiration was at once arrested. The second injection (*b*) at 3.7 p.m. The respiration was arrested. The third (*c*) at 3.9 p.m. The respiration became slower and deeper but was not arrested. The fourth (*d*) at 3.12 p.m. The respiration was slightly slowed. The fifth (*e*) at 3.13 p.m. Respiration not affected. The animal was now kept under the anæsthetic for 40 min. and then a sixth injection made (*f*) at 3.53 p.m. Respiration was slowed but not arrested. A seventh injection (*g*) was made at 3.55 p.m. Respiration unaffected.

the injections the more pronounced was this result. The reaction which first disappeared was nearly always that upon the respiration. Either the respiration was only slowed and diminished in depth, or in other cases no effect was produced. Later the effect upon the heart became modified, the inhibition being less marked and lasting a shorter time, until in still later injections no action upon the heart could be distinguished. Even in these cases however some of the effect upon the vaso-motor centre was still observable, though this too was of smaller extent.

The tracings (Fig. 14 *a*—*e*) show the result upon blood-pressure of a series of five successive injections. These were made at short intervals and it is seen from the tracings that the reaction gradually disappears. There was then an interval of 40 minutes, at the end of which an injection produced the result seen in Fig. 14 *f*. Thus, during this period of rest the animal had to a considerable extent regained its first condition as to this reaction, but it is seen from the next tracing (Fig. 14 *g*) in which a further injection of serum was made two minutes later that it very soon returns to its non-reactive state.

Injection of serum therefore sets up a condition of immunity. This immune stage lasts for some time and is followed by a second in which a single injection at once causes a return to the immune condition. The length of this second period extends, so far as my present observations have shown, at least two hours. I have not been able to observe any alterations in the blood during this period either with respect to its rate of coagulation, or in its corpuscles. The condition of immunity is apparently due to an alteration in excitability of the pulmonary nerve endings.

As previously mentioned there are several other bodies, chiefly of a proteid nature, which produce the same result as serum when injected into the veins of a cat, and I have similarly found that they also are able to produce an analogous immune condition. Further, I have found that if immunity be produced by one substance, that animal also gives, as a rule, no reaction to the other substances which act similarly.

The other two animals I have tested, viz. the dog and the rabbit, are naturally immune: The reaction given by a dog for instance very closely resembles that obtained in the cat when both vagi have been divided in the neck (Fig. 2). An injection of serum into a dog causes a slight rise of blood-pressure lasting about 10 seconds and followed by a more marked but still slight fall which lasts about 25 seconds[1]. There is no alteration in the rate of heart-beat nor in the respiration unless very large doses are injected. Section of both vagi in the neck in no way modifies this reaction.

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[1] The variations are exactly like those reproduced in Fig. 5.

IV. WHICH CONSTITUENT OF SERUM IS IT WHICH PRODUCES THE REACTION?

The second series of experiments in connection with this investigation were devised with the object of ascertaining whether there was only one constituent in serum which was the active body, and if so what that constituent was.

In attempting to determine this the following points were proved:

(*a*) *The active substance is a proteid.* It was found that, if serum was boiled and the resulting coagulum filtered off, the filtrate gave no reaction. It was thought that possibly the active substance was the nucleo-proteid of serum. To test this, serum was diluted five-fold and weak acetic acid added. The resulting precipitate of nucleo-proteid and globulin was collected, dissolved in a small bulk of 1 °/₀ sodium chloride, and the solution injected. It was found to be quite inactive, while the filtrate from the original precipitate was as active as before precipitation.

(*b*) *The active substance is a proteid of the albumin class.* This was ascertained by half-saturating serum with ammonium sulphate and filtering off the precipitated globulin, which was then dissolved and again precipitated. This second precipitate was collected, dissolved and injected with negative result. In a control experiment, the injection of ammonium sulphate of corresponding strength was proved to produce no result. Some of the filtrate from the globulin precipitate was diluted, in order to lower the percentage amount of ammonium sulphate, and then injected. It gave the usual reaction. The rest of the filtrate was therefore saturated with ammonium sulphate and the precipitated albumins collected, and dissolved. This solution was found to be active, whereas the filtrate was quite inactive.

The active substance is therefore to be found among the albumins. A further difficulty was however met with here. For the purification of the albumins, solution and precipitation was repeated four times. The final solution was found to be nearly inactive. The filtrates obtained in the process of purification were therefore dialysed but were found to be quite inactive. Dialysed serum is however just as active as the original serum. The addition of salts to the purified albumin solution produced no effect on the general result. Hence it seems that the active material is some more complex combination of the albumin, and that it is broken down by repeated precipitation with ammonium sulphate.

(c) *The active substance is coagulated at a temperature of* 78° C. In order to determine the temperature at which the active substance is destroyed, serum was diluted with an equal volume of water, rendered just acid with acetic acid and heated. It was kept at a definite temperature two minutes, filtered if necessary and injected. These filtrates were always active until those were reached which had been heated to or above 78° C.

A further experiment of this class was carried out for the following reason. As previously mentioned (p. 49) Friedenthal and Lewandowsky found that if serum be heated for an hour to 58° C.—60° C. it loses its toxic properties. In order to test whether the substance producing the reaction described in this paper is the same as that which kills an animal when subcutaneously injected I treated serum in the manner they describe and then injected it. A typical reaction was however produced (Fig. 15), so that the two substances are apparently different.

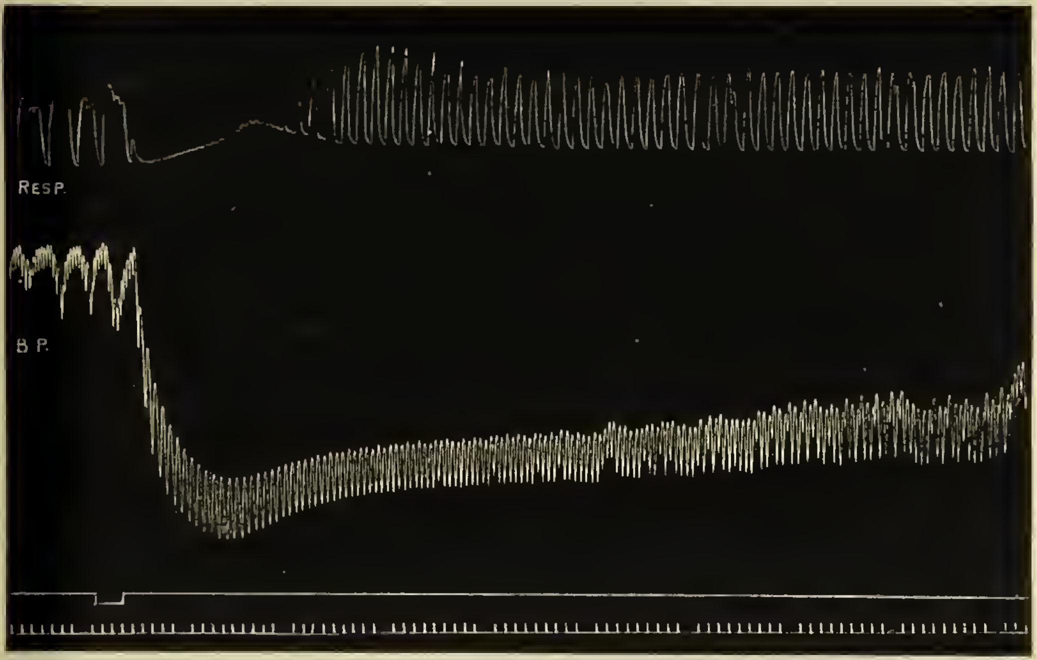

Fig. 15. Cat under urethane. Respiration and blood-pressure. The prolonged inspiration seen at the beginning of the record is due to the urethane. At the signal 4 c.c. of cat's-serum, which had been previously heated to 59° C. for one hour, was injected.

(*d*) *The active substance is produced during the process of clotting.* Having found that serum from any animal, including the cat, was active I determined to try whether whipped blood taken from the same animal would also prove active. The animal being therefore prepared in the usual way, a sample of blood was collected from the carotid. This was whipped and immediately injected. It gave a perfectly typical reaction (Fig. 3). The time elapsing between the collection of the blood and its injection into the vein never exceeded two minutes. Having obtained this rather surprising result I then prepared the opposite carotid, tied in a cannula and connected it to the cannula in the jugular vein. The blood from the artery was then allowed to flow directly into the vein with completely negative results. Hence it follows that the active substance is only produced during the process of clotting. This fact was further corroborated by many other experiments as will be presently described. The result suggested that the active substance might be the proteid split off from fibrinogen during fibrin formation. The one body at present known is Hammarsten's fibrino-globulin. This however is a body of the globulin class, clotting at 64° C. and therefore disagreeing markedly with the properties previously found for the active substance.

(*e*) *The corpuscles take an active part in the formation of the active substance.* After finding that the active substance was only produced on the clotting of blood, I thought that further attempts to isolate it would be simplified by working upon plasma. I therefore prepared samples of magnesium sulphate plasma, sodium sulphate plasma and sodium citrate plasma. All these proved to be inactive. It was surprising however to find that the serum prepared from these plasmas was, in nearly all instances, also inactive.

Samples of the plasma were therefore taken and in the case of the salted plasma one sample was simply diluted and allowed to clot, a second was diluted, some of the corpuscles added and then this also allowed to clot. In the case of the citrated plasma one sample was treated with calcium phosphate and brought to body temperature, a second sample being mixed with some of the corpuscles, and then, by similar treatment, made to clot. In the majority of such experiments it was now found that the serum from the pure plasma was inactive, whereas the serum from the plasma admixed with corpuscles was as a rule active. In a few experiments when adding the corpuscles those were chosen which formed the upper layer of the sediment of corpuscles and contrasted with a similar sample taken from the lowest layers.

I was not however able to make out any difference in the behaviour of the serum obtained, although microscopic examination proved that the upper layers were much richer in white corpuscles and in blood-platelets. Although from the many experiments of this kind that I have made, I feel sure that the statements just given are in the main correct, yet I have met with several exceptions which have proved very puzzling. Thus at times a serum prepared from the plasma above has proved active, and on the other hand a serum formed from a plasma mixed with corpuscles has on a few occasions proved inactive. I have never found a serum or a defibrinated blood obtained in the ordinary way which has proved inactive. I have also tested hydrocele fluid. The sample I obtained had clotted before I received it. The clot was quite colourless, there was no sign of any admixture with blood, yet the serum when injected proved typically active. Unfortunately neither the clot nor the serum was examined for white-corpuscles and platelets.

In regard to these latter points the experiments are incomplete and though I contemplate continuing them in this direction, other work prevents their renewal now, and I have therefore thought it best to publish the results as they at present stand.

CONCLUSIONS.

1. Intravenous injection of blood-serum from any source into a cat causes arrest of respiration, inhibition of the heart and vaso-dilatation. The effects persist for some time.
2. These effects are reflex, and are almost entirely absent if the vagi be previously divided in the neck.
3. The effect is due to excitation of the pulmonary nerves. It is cut out by division of the pulmonary branches of the vagus, not by division of the cardiac or lower branches.
4. Repetition of the injection leads to the production of an immune state.
5. The active substance in the serum is a proteid. It is of the albumin class and is coagulated on being heated to 86° C.
6. The active substance is only produced when the blood clots.
7. The interaction of the blood corpuscles is a necessary condition for its formation. Serum obtained from plasma is inactive.

[Part of the expense of this research has been defrayed by a Grant from the Government Grant Committee of the Royal Society.]

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THE POSITION OF THE RESPIRATORY AND CARDIO-INHIBITORY FIBRES IN THE ROOTLETS OF THE IXth, Xth AND XIth CRANIAL NERVES.

By A. W. CADMAN, F.R.C.S. *Lecturer and Demonstrator in the Anatomical Department of King's College, London.*

(*From the Conjoint Laboratories of the Royal Colleges of Physicians and Surgeons, London.*)

THE experiments which are described in this paper have been undertaken with the object of determining the functions of the rootlets of the ninth, tenth and eleventh cranial nerves as they leave the medulla. Although anatomically arranged in three groups, this grouping, as is well known, does not correspond to the three large nerve trunks as they issue from the base of the skull.

The present paper deals with three sets of fibres only, viz., the respiratory (Hering-Breuer) fibres, the efferent cardio-inhibitory fibres, and the afferent fibres of the vagus excitation of which causes reflex cardiac inhibition.

Cats and dogs were chosen for the experiments, and they were throughout anæsthetised with the A.C.E. mixture. The animal having been anæsthetised, the two carotids were exposed, one was ligatured, and a cannula was tied in the other so that the blood-pressure could be subsequently recorded. A cannula was next tied in the trachea. Loops of thread were passed round the two vagi in the neck, the vagi being separated from the cervical sympathetics. The ligature of both the carotids prevented a great deal of the bleeding in the later stage of the operation while the circulation in the brain and medulla was little affected. The medulla was always found to be quite sensitive and to give good reflexes.

The animal was now turned over, the left temporal muscle was reflected and the muscles of the back of the neck removed on either side so as to thoroughly lay bare the occipital bone, occipito-atloid ligament and atlas. In reflecting these muscles care must be taken to ligature a large artery and vein issuing with the first cervical nerve. The occipito-atloid ligament was cut in the middle line and the dura mater separated from the lower margin of the supra-occipital bone. The whole of the supra-occipital, and a small part of the posterior border of the left temporal bone was then rapidly removed. Considerable bleeding takes place from the sinuses as they are cut across. This is at once checked by having the head of the animal raised well up above the trunk, and the sinuses are then plugged with wax. The skull was cut freely away laterally; the bleeding at each cut being stopped as before. Great care is taken throughout this stage that the dura mater is not injured. Sufficient bone having been removed to allow of free exposure of the rootlets, the dura mater was incised in the middle line and thrown down towards the left side. Upon now gently displacing the cerebellum the rootlets of the three nerves can be brought into view. The rootlets lie at the bottom of a deep conical space in which the blood, from the torn small vessels, collects and effectually prevents a view of any single rootlet sufficiently distinctly to allow of its section. To avoid this difficulty small pledgets of cotton wool with clean cut edges were prepared of such a size as to fit the space: these were inserted and pushed gently against the cerebellum and medulla. The pressure necessary to push aside the cerebellum at the same time stops the bleeding which occurs from this surface, and, in this way, the rootlets can be brought into clear view and divided as necessary.

At first it was attempted to determine the functions of the rootlets by exciting them directly with induction shocks, but this proved most unsatisfactory as, in the first place, the working space is so contracted, and secondly, the rootlets lie so close together, that it is impossible to prevent escape of current to other rootlets than the one excited. Excitation reflexly was, therefore, employed in all the later experiments. The plan of the experiment was to obtain a reflex through the rootlets, and then to cut a single rootlet and note if the reflex had been in any way affected.

The rootlets, as is known, are arranged in three groups[1]. In the cat the uppermost consists firstly of one large rootlet, and this is followed by one, or sometimes two, rather smaller rootlets. The second group is made up of a series of about eight to ten small rootlets which follow each other closely forming a band-like arrangement: these lie on a plane a little posterior to the upper group. The third group generally consists of three distinct rootlets situated in the angle between the spinal portion of the spinal accessory nerve and the medulla. These will be referred to in this paper as the first, second, and third groups respectively.

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[1] Cp. Kreidl, *op. cit. infra* and the papers there quoted.

In the dog the arrangement is much simpler. All the fibres leave the medulla in three large rootlets, which correspond to the groups of rootlets found in the cat. The three big rootlets are also well separated from one another.

SECTION I. *The respiratory fibres.*

In order to determine the rootlets in which these fibres enter the medulla, experiments were carried out in the following manner. The rootlets having been exposed on the left side of the medulla, the right vagus was then divided in the neck.

The left uncut vagus was now excited by weak induction shocks to show that it produced arrest of respiration. After recovery from this excitation the rate of respiration was counted. The uppermost bundle of the first group was now divided and any effect on the rate of respiration recorded. Similarly the rootlets were divided one by one from above downwards, watching for the slowing of respiration which follows division of both vagi in the neck. After each rootlet was divided the left vagus was excited to see if any effect upon respiration resulted. There were therefore two signs indicating that the respiratory fibres had been divided, viz.: (i) the slowing of the respiration and (ii) absence of effect on respiration when the left vagus was excited in the neck. By this experiment the lowest limit at which these fibres enter the bulb was determined.

By an exactly similar procedure, in other experiments, but starting with the lower rootlets and passing upwards, the upper limit by which the fibres enter was determined. Finally the experiment was made of picking out those rootlets only which lay between the limits determined by the earlier experiments.

In this way it was proved that the afferent respiratory fibres enter the bulb in the lower rootlets of the upper group. If there are two rootlets here, both contain respiratory fibres. If there is but one rootlet, all these fibres lie in that rootlet. Respiratory fibres were never found in the upper rootlet. Division of the rootlets of the second and third groups have no effect whatsoever upon respiration.

The arrangement in the dog is exactly similar. All the respiratory fibres enter in the upper rootlet.

SECTION II. *The efferent cardio-inhibitory fibres.*

The general plan of the experiments for tracing these fibres in the rootlets was as follows. When the rootlets had been exposed upon the left side the animal was placed on its back and the cannula in the left carotid connected to a mercury manometer. The right vagus was then divided in the neck and its central end excited electrically. The blood-pressure tracing gave the record of the cardiac inhibition. The animal was now turned over and the uppermost rootlet divided. The central end of the right vagus was again excited. The rootlets were now divided seriatim from above downwards and reflex cardiac inhibition watched for after each division. In this manner the lowest limit at which these fibres leave the bulb was determined. In the next animal the uppermost limit was determined, and, finally, by picking out the particular rootlets, the general result confirmed.

In this way it was shown that the efferent cardio-inhibitory fibres of the vagus leave the bulb in the two lowest rootlets of the third group. They are never contained in the upper rootlet or rootlets of this group, and, if there are only two rootlets present, the fibres are all contained in the lower one. In the dog the arrangement is the same; all these fibres run in the lowest rootlet.

SECTION III. *The afferent fibres excitation of which causes reflex cardiac inhibition.*

The course of this set of fibres was determined by an analogous set of experiments. The left vagus being cut in the neck, its central end was excited before and after division of the various rootlets. The absence of effect on the blood-pressure record gave the limits of the rootlets which carry these fibres into the bulb. It was thus proved that these fibres entered by the lower two rootlets of the first group. In fact, the entrance of these fibres is exactly the same as that of the respiratory fibres. This result, therefore, falls well into line with the results obtained by Brodie and Russell[1] on reflex cardiac inhibition. They show that the branches of the vagus excitation of which results in cardiac inhibition are the pulmonary branches. Stimulation of the central end of the divided cardiac branches commonly produces no effect on the heart rate, sometimes it is slowed, but even then the inhibition is never so pronounced as that following stimulation of the pulmonary nerves. Similarly excitation of the vagi below the point at which the pulmonary nerves are given off frequently causes no inhibition and when the latter is produced it is never very considerable.

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[1] See this number of the *Journal, infra.*

Observations on the function of the rootlets have been made by several observers. Grossmann[1] carried out his experiments upon rabbits, in which animal the arrangement of the rootlets is practically the same as in the cat. The occipito-altoid ligament was exposed and opened. No bone was removed. The rootlets were excited electrically and the effect upon the heart determined by recording the blood-pressure. Inhibition was only obtained when the lowest rootlets of the middle group and the highest of the lower group were excited. These results differ from those I have obtained upon the cat. I have not as yet attempted to confirm these results by the reflex method upon the rabbit. In the cat it is quite impossible to see the rootlets unless the bone be removed. Stimulation of the rootlets electrically is I think very untrustworthy for this purpose. While employing it I never obtained the precise and definite results obtained by the experiments above described.

Kreidl[2] also worked upon rabbits. Among other results he observed, he describes slowing of respiration as following the tearing through of all the rootlets of the upper group. His results therefore agree with mine, but he was not able to discriminate between the different rootlets of that group.

Beer and Kreidl[3] have employed a method which is practically the same as mine. Among other fibres they worked out the course of the respiratory and describe them as entering in the lower rootlets of the upper bundle. My results upon cats and dogs, therefore, agree very well with theirs upon the rabbit.

In a later paper Kreidl[4] gives the results of some experiments of the same kind upon monkeys (*Macacus Rhesus*). The rootlets were exposed and excited electrically. He found that the inhibitory fibres

[1] Grossmann. *Pflüger's Archiv*, LIX. p. 1. 1895.

[2] Kreidl. *Pflüger's Archiv*, LIX. p. 9. 1895.

[3] Beer and Kreidl. *Pflüger's Archiv*, LXII. p. 156. 1895.

[4] Kreidl. *Sitz. d. k. Akad. Wien*, CVI. p. 197. 1897.

for the heart left the bulb by the upper two rootlets of the middle bundle. The respiratory fibres enter by the lower rootlets of the upper group. As far as the respiratory fibres are concerned, my results upon the cat are in agreement with his upon the monkey. As regards the cardiac fibres our results differ materially. In my opinion the method adopted by Kreidl for determining the course of these fibres is not so satisfactory as the reflex method I have adopted. I have found that these fibres enter in quite the same position in both cat and dog, and in a successful experiment the answer given by the reflex method is very decisive.

I wish here to express my thanks to my friend Dr T. G. Brodie for his very valuable hints and great help in these experiments.

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NOTES ON THE TOXICITY OF DIFFERENT SPECIMENS OF THE BACILLUS COLI COMMUNIS, OBTAINED FROM VARIOUS SOURCES.

NOTES ON THE TOXICITY OF DIFFERENT SPECIMENS OF THE BACILLUS COLI COMMUNIS, OBTAINED FROM VARIOUS SOURCES.

By Vincent Dormer Harris, M.D., F.R.C.P.

(*From the Laboratories of the Royal Colleges of Physicians and Surgeons, London.*)

During the last ten years or more, considerable interest has been evinced, both by bacteriologists and clinicians, in the *B. coli communis.* In the first place, its considerable likeness to the *B. typhosus* (Eberth's bacillus), in regard to its morphology and biology as well as its association with the specific bacillus in typhoid dejecta, renders its certain detection of much importance; secondly, its appearance in almost pure culture in the dejecta of cholera, cholera nostras, infantile diarrhœa, and in enteritis, which marks its close relation with the production of these affections; thirdly, its very frequent presence in the inflammatory exudations, not only in connection with the peritoneum, but also elsewhere in the human body, which suggests its causal influence in certain conditions; and lastly, as it is an invariable constituent of the fæces of man and probably of other animals, its isolation from waters, at any rate in considerable amount, has been taken to indicate sewage contamination.

It is not surprising, therefore, that many investigators should have turned their attention to the study of this micro-organism. As a result of their researches, some of which have been most exhaustive and valuable,[1] the differences between the *B. coli* and the *B. typhosus* are fairly well established, not so much by the observation of minute differences in the appearances and methods of growth of the two micro-organisms, although these are of value as secondary differences, as by two chemical reactions, namely, that the *B. coli* produces indol in peptone broth in a comparatively short time when incubated at about 37° C., and that it also in much the same time causes the precipitation of caseinogen in milk, by the fermentation of lactose, whereas the *B. typhosus* produces neither of these phenomena. Some other interesting points of divergence, *e.g.* the refusal of *B. typhosus* to grow in broth which contains even a minute trace of arsenious acid, whilst the *B. coli* will grow in a fairly strongly impregnated arsenious acid broth, and the now well-known and clinically-used "Serum Reaction," need not detain us. Having, however, separated the *B. typhosus* by the two main tests just mentioned from other similar organisms associated with it in the intestinal contents and elsewhere, and which are grouped together under the same name, *B. coli*, we are at once met by the fact that although such organisms possess many properties in common, they differ among themselves in certain points. The important question that naturally follows is as to whether these differences are mere non-essential qualities allowable to members of the same species or whether they are of sufficient importance to determine different species. Unfortunately this question cannot at present be answered definitely, and a very superficial examination of the considerable literature upon the subject is sufficient to indicate the very different views taken as to what are and what are not the fundamental qualities of the *B. coli*. All that seems certain about the matter is that from intestinal mucus, from fæces, from sewage, from water, from milk, from contaminated meat, from inflammatory exudations and elsewhere, micro-organisms called by the same name (*B. coli*) can be isolated, which in many respects closely resembling the *B. typhosus*, but differing from it in the manner spoken of above, show considerable differences among themselves.

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[1] The general summary of which, up to the date of writing, by R. Wurtz (*Arch. de méd. expér. et d'anat. path.*, Paris, 1893, pp. 131–162) is of great interest.

It may be as well to summarise shortly some of the differences which have been acknowledged to exist between different specimens of the *B. coli* obtained from different sources. First then, as to its *shape and size*; it may vary from micrococcus to true bacillus type, with ends more or less rounded, in length from 2 to 4 μ or more. These differences may or may not be entirely or partially due to the age of the specimen, as suggested by some, or to the differences in the nutrient material, as suggested by others. My own view is that although both of these agencies may enter into the production of the different appearances, yet some of the bacilli are essentially smaller than others from their original isolation, and that age of growth and nature of nutrient material are only secondary influences. Secondly, as to *motility*; extraordinary differences are observable as regards the motility of the bacillus. In some cases, one might imagine one had to deal with a perfectly immotile organism, in others the motility is as lively as that of the *B. typhosus*, or nearly so. No doubt this difference may be more apparent than real, unless it is perfectly certain that growths of the same age are compared. Naturally, the same remark applies to the flagella, and although it may be true that many specimens of the bacillus show only two cilia, yet some are hardly to be differentiated from the *B. typhosus*. From a somewhat prolonged investigation of this question, I am inclined to think that the flagella of the *B. coli* are somewhat more delicate than those of the *B. typhosus*, but frequently there is little difference to be distinguished as regards their great length and their corkscrew-like appearance. At the same time it must be conceded that the amount of motility in different specimens is a variable factor. Thirdly, *on potato*, the growths differ in the amount of colour from white tinged with yellow to yellow-brown, and in the amount of the heaping up of the growth above the surface. The growth on potato was formerly considered more important than it is to-day, but minute differences can be recognised. Fourthly, the *production of gas* in nutrient media; this certainly varies considerably. In some cases a peptone-gelatin shake-culture will, in a day or two, present the appearance of masses of crystallised sugar, a very beautiful and characteristic phenomenon, whereas in other instances there is little, slower, or no production of gas. These differences appear to be too striking to be accounted for by differences in the composition of the nutrient material prepared after the same formula. This same remark applies also to (fifthly) the *reaction* of the nutrient substance, as shown in litmus-gelatin. There is no doubt but that, although it may be the majority of specimens produce on such a medium an acid, some produce the opposite or an alkaline reaction. Sixthly, the *opalescence of the nutrient material*, the metallic lustre about the growth in stroke cultures and the arborescences appearing laterally in the gelatin in stab cultures. Seventhly, as to the *substances produced in nutrient broth* by the growth of bacillus; this is too wide a subject to be treated of except in very general terms, but it is certain that, for example, whereas all the specimens of so-called *B. coli* in my hands (whenever tested) produced in a very few hours lactic acid in broth containing sugar, butyric acid sometimes also may be produced under the same conditions in conjunction with lactic, but very infrequently. Lastly, as regards the *time required* by different specimens *to produce indol in peptone broth* and *to precipitate milk*. Under exactly the same apparent conditions some specimens produce their effects much more rapidly than others, and as regards the effect upon milk, although generally the curdling is simply precipitation of caseinogen (as the precipitate can be redissolved), in other cases there seems to be a ferment action, and a true clot seems to be formed. I will not touch upon differences which may be noticed as regards growth of the *B. coli* in nutrient media to which different proportions of phenol or arsenious acid have been added, of which I have not had personal experience. As regards all the above-mentioned and other differences between different specimens of the *B. coli* obtained from different sources, it seems to me that they might reasonably be considered as non-essential, but I am unable to regard the variation in the toxicity of different specimens in the same light. Of these variations I have no doubt, and the subjoined account of my experiments on the subject will, I think, confirm this conclusion.

Of the experiments.—I commenced a series of experiments several years ago, to discover whether one could obtain a certain test for the *B. coli* by its effect upon animals when inoculated in increasing doses. I devoted much time to the thorough investigation of the specimens of the *B. coli* I had from time to time obtained from different sources, and rejected all those examples which did not satisfy the three tests, namely, that it should be a non-liquefying organism,[1] growing in a certain way, which in a day or a day and a half produced indol in peptone broth, and curdled milk when incubated at about 37° C. Those organisms, for several of which I have to thank friends,[2] which satisfied these requirements were used to inoculate guinea-pigs and rabbits, broth cultures of about two days' growth, or gelatin, or agar cultures rubbed up in water or saline solution being chiefly employed for the purpose. The results of my experiments were so conflicting, some of the cultivations appearing so toxic, whilst others seemed to be so much the reverse, that I for a time gave up the experiments as being too contradictory to be of any use. More recently, finding that the experiments of others have shown much the same irregularity, I have added to my former series. If for no other purpose than to show negative observations, I have been advised that it is worth while to put some account of my experiments on record. I am inclined to think, however, that my results point to a more definite conclusion; but to this I propose to return later.

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[1] Occasionally one meets with a specimen of the so-called *B. coli* which, after some time, many days or even weeks, gradually liquefies gelatin. I have had several of such specimens given to me, but have not included them in my experiments.

[2] And in this connection I would particularly mention Professor G. Sims Woodhead, Drs. Cantlie and S. Fenwick, and Messrs. Bokenham and Alex. G. R. Foulerton.

Of micro-organisms which are supposed to be identical with B. coli.—Several micro-organisms which have been isolated from the fæces or from intestinal mucus, or from the dejecta in various cases, and which have received different names from their discoverers, are now considered to be identical with the *B. coli*; of these I may mention particularly two, namely, the *B. neapolitanus* of Emmerich, which was at first supposed to be the specific micro-organism of cholera in an outbreak at Naples, and the *B. cavicida* (Brieger's bacillus), first isolated from human fæces, as the series includes experiments made with these micro-organisms.

Sources of the micro-organisms experimented with.—The different specimens of the *B. coli* experimented with include (*a*) four different specimens obtained from the Continent—they were *B. neapolitanus*, *B. cavicida*, and two called *B. coli*, one from Munich and the other from Paris; (*b*) three specimens which were isolated from intestinal mucus, one each from that of the human subject, rabbit, and guinea-pig; (*c*) five specimens which were isolated from the dejecta in cases of epidemic diarrhœa, one from infantile diarrhœa, and four from dejecta in diarrhœa epidemics in different parts of the country; (*d*) one specimen was obtained from suspicious oysters; (*e*) five specimens which were isolated from specimens of water from different parts of the country; (*f*) six specimens which were obtained from fæces or sewage; (*g*) one specimen from suspicious milk; (*h*) five specimens which were isolated from abscesses in the human body.

Plan of experiments.—As above indicated, the cultures of the *B. coli* experimented with were grown in two ways, namely, on gelatin, or on agar for about a couple of days, and in peptone broth, for about the same duration of incubation. In a few cases the toxines obtained from the broth cultures were tested. The inoculations were either intraperitoneal, intravenous, or subcutaneous. In the early experiments all the three channels were used, but experience showed this to be unnecessary. The greatest effect, and the most rapid, was produced when the micro-organism was introduced into the peritoneum, but when introduced into the veins the effect was practically the same but rather more delayed, and to produce the same effect subcutaneously a much larger dose was required (three or four times at least), and the delay was greater. If, therefore, a dose of the cultivation did not produce an effect when introduced into the peritoneum, it was assumed (not in all cases however) that it would produce negative results, intravenously and subcutaneously. The gelatin or agar culture was considerably more effective than the broth culture, and the effect of the toxin when tried was exceedingly feeble. It is difficult to exactly indicate the dose when a gelatin culture (or an agar culture) is used, and the expression with regard to this "small dose" must be considered a minute proportion of a single tube. As regards the dose of the broth cultures, anything more than 2 c.c. would be considered a large dose, and 1 c.c. or below this a small dose. Above 3 c.c. might be called a very large dose. I have had little experience, in this series, of intrapleural inoculations, which are said to be the most effective of all, but as the two serous cavities are in direct communication it appears unlikely that there should be any great fundamental difference between the effect if the pleura were infected instead of the peritoneum. In many of the cases tested the bacillus soon appeared in the blood.

Without going into detail with the experiments, which would be not only tedious but also unnecessary, I will take the results *seriatim.*

In the first group may be included the four specimens, which were obtained from the Continent.

1. *B. neapolitanus* (Emmerich).—The specimen used was a remote subculture, which had been kept up in the Colleges' Laboratories for some years. In morphology, growth, and chemical reactions it proved to be a very typical example of the organism, and singularly like the description given in his original communication by Emmerich. In spite of its long line of ancestors, the specimen showed marked toxic powers, being fatal both to rabbits and guinea-pigs in small doses. 1 c.c. of a two days' old broth culture, after filtration through filter paper, produced fatal effects in rabbits (about 1500 grms. in weight) in about twenty-four hours if injected into the peritoneum, and if into the veins in about thirty hours. Gelatin or agar cultures, in small doses, produced much the same effect. When the dose was doubled or trebled no more rapid death took place. The specimen was extremely fatal to guinea-pigs in minute doses, causing death in about twenty-four hours.

The toxine, in large doses, was never fatal either to rabbits or guinea-pigs, but generally produced considerable rise of temperature, drowsiness, loss of appetite, and diarrhœa, sometimes with blood. Very much the same effect was produced wherever the material was introduced, but, naturally, the greatest was when the injection was made into the peritoneum.

2. *B. coli communis* (Escherich), *remote subculture obtained from Munich.*—This organism in mode of growth, in chemical reactions, and morphology might be considered a typical *B. coli.* More than any of the other specimens experimented with, it showed in a stab culture the beautiful lateral arborescences, moss-like tufts appearing along the line of growth (well figured by Sternberg[1]) with the opalescence of the gelatin in oldish cultures. This organism was tested on both rabbits and guinea-pigs, and even in large doses did not produce death. Neither the growth upon gelatin nor yet the broth culture, even when introduced into the peritoneum in large doses, produced any greater effect than malaise and drowsiness, which quickly passed off. In one case injected with a large dose of a gelatin culture, considerable sloughing of the skin ensued. In this particular instance, as the culture was a remote one from the original,

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[1] "Bacteriology," p. 442.

it is possible that by continued re-inoculations it had lost its virulence, but the same remark applies also to (1), which was also a remote subculture, and yet retained to a marked degree its toxic power. In experiments with this specimen of the organism a considerable part of two gelatin cultures of the usual size did not produce death when injected into the peritoneum of a medium-sized rabbit.

3. *B. coli*, from Paris.—This organism, presenting the usual appearance of the typical growth, was found to curdle milk very rapidly, closely resembled (2), and was no more toxic. It is interesting to remark that this organism was one of a very few which produced an appreciable trace of butyric acid in a broth culture with glucose. Unfortunately, the exact source of the bacillus cannot be given.

4. *B. cavicida* (Brieger).—This again was a remote subculture. It proved to be very fatal to guinea-pigs but was innocuous to rabbits. This corresponds very much to Brieger's experience of the micro-organism. Sternberg writes of his experience of the same microbe. He supposed that the pathogenetic power of the bacillus had become attenuated, inasmuch as considerable quantities of a pure culture injected into guinea-pigs did not cause death. The culture used came originally from Germany.

The second group includes specimens of the *B. coli*, isolated from intestinal mucus.

5. *B. coli, isolated from the intestinal mucus of the human intestine.*—It is well known that the *B. coli* may be isolated from the mucus in any part of the intestine, and particularly from that of the upper part of the tract (the duodenum). This specimen was isolated from a case in which death had been caused by disease not in connection with the alimentary canal, which was apparently healthy. Small doses of this bacillus were innocuous both to rabbits and guinea-pigs. Large doses appeared to be more toxic to rabbits than guinea-pigs, producing death in those animals but not in guinea-pigs. The gelatin cultures were much more toxic than the broth cultures.

6. *B. coli, isolated from the intestinal mucus of the guinea-pig.*—In this case the cultures used were rather later than in other cases, *i.e.* four days instead of two to three. A medium dose introduced into the peritoneum of the guinea-pig produced death in twenty to twenty-four hours. The whole of the peritoneum was very inflamed, and the stomach, liver, and spleen were covered over with shreddy lymph. There was intense injection of the intestines, which were also distended. Fluid in peritoneal cavity. Venous system and heart were distended with blood; no clotting. Small hæmorrhages into walls of stomach. There was no permanent effect produced with small doses or from subcutaneous injection. Medium doses were not fatal to rabbits, but produced malaise and drowsiness. Broth cultures produced much the same effects as gelatin or agar cultures.

7. *B. coli, isolated from the intestinal mucus of the rabbit.*—This bacillus was, in all the cases tried, innocuous both to guinea-pigs and to rabbits.

The next group (*c*) comprises specimens obtained from alvine evacuations in cases of cholera nostras or epidemic diarrhœa, one being from a case of infantile diarrhœa and the other four from adult cases.

8. *B. coli, from the alvine evacuations in a case of infantile diarrhœa.*—This specimen was, in every case tried, non-fatal to both rabbits and guinea-pigs from whatever culture. In one case a very large dose injected into the veins of a rabbit produced a condition of watery evacuations, but this passed off.

9, 10, and 11, all obtained from cases of cholera nostras, were very much alike in their action, being extremely fatal to guinea-pigs and not fatal to rabbits, in fact they closely resembled in their effects *B. cavicida.*

12 was also very fatal to guinea-pigs, and in large doses fatal to rabbits.

The next specimen was obtained from suspicious oysters.

13. *B. coli, from suspicious oysters.*—This specimen proved to be, as far as my experiments went, absolutely innocuous to guinea-pigs as well as to rabbits.

The next group (*e*) comprised five different specimens (Nos. 14, 15, 16, 17, and 18), which were isolated from five different samples of water from different parts of the country.

14, 15, 16, 17, and 18. *B. coli, from different samples of water.*—The first four of these specimens were, as far as could be made out, totally innocuous to rabbits and guinea-pigs. Specimen No. 18, however, was very toxic to guinea-pigs, small doses producing death in about twenty-four hours. It was not fatal to rabbits, but produced in them marked malaise, which passed off.

The next group comprised three specimens obtained from fæces.

19, 20, 21. *B. coli, isolated from fæces.*—All of these specimens were innocuous both to rabbits and guinea-pigs. One specimen, No. 21, was made up of bacilli more lively than any others examined as to their movements. They were provided with flagella which seemed to envelop the rods.

The next group (*f*) comprised specimens obtained from sewage.

22, 23, 24. *B. coli, isolated from sewage.*—All of these specimens were non-lethal both to rabbits and guinea-pigs.

Next we have to do with one specimen from suspicious milk.

25. *B. coli, isolated from suspicious milk.*—It proved on examination to be innocuous both to rabbits and guinea-pigs.

The last group comprised five specimens isolated from abscesses in the human body.

26, 27, 28, 29, and 30. *B. coli, isolated from abscesses in the human body.*—These specimens are of very great interest. No. 26 was isolated from the pus of a suppurating ovarian cyst which was connected with the rectum. Death resulted in three days after commencement of illness. The bacillus was also isolated in pure culture from the blood of the heart, the peritoneal fluid, and the organs of the cadaver. The organism proved to be very toxic both to rabbits and to guinea-pigs. A broth culture of twenty-four hours' incubation, in doses of 5 c.mm., was lethal both to rabbits and guinea-pigs, whether introduced into the veins or intraperitoneally. Death resulted, in the case of guinea-pigs, in about twenty hours, and in the case of rabbits in two or three days. No increase of the dose appeared to produce any increase in the rapidity of the effect. In all of the fatal cases pure cultures were to be obtained from the blood and organs of the animal. The local or peritoneal effect was not excessive, beyond distension of the abdomen, and some lymph in the peritoneal cavity. The smallest dose definitely found to be fatal was, as above, 5 c.mm., as 2·5 and 1 c.mm. were found not to produce death in the animals experimented with. In these experiments the want of severity of the local lesions was very noticeable.

28 was from a case of ovarian abscess, as was also 29, whereas 30 was from an abscess in connection with the gall bladder. All three specimens were very fatal to guinea-pigs in small doses, death resulting in about twenty-four hours. They were not all equally fatal to rabbits.

Table of Experiments with Results.

Micro-organism experimented with.	Source.	Fatal to Guinea-pigs.	Fatal to Rabbits.	Non-fatal to Guinea-pigs.	Non-fatal to Rabbits.	Remarks.
1. *B. neapolitanus* (from abroad).	Epidemic cholera.	Very, in small doses.	Very, in small doses.	...	...	Although an old culture, retained virulence.
2. *B. coli* (Escherich).	Fæces; obtained from the Continent.	...	...	Yes.	Yes.	Characteristic growth; no toxic growth retained after many generations.
3. *B. coli* (Paris).	Unknown; obtained from Paris Laboratory.	...	...	Yes.	Yes.	Recent culture.
4. *B. cavicida* (Brieger).	Fæces.	Very, in small doses.	...	...	Yes, even in large doses.	Although cultivated through many generations, retained its power.
5. *B. coli*, from human intestine.	Intestinal mucus.	...	Yes, in large doses.	Yes.	...	
6. *B. coli*, from guinea-pig.	Intestinal mucus.	Yes, in medium doses.	...	...	Yes.	
7. *B. coli*, from rabbit.	Intestinal mucus.	...	...	Yes.	Yes.	
8. *B. coli* (human).	Dejecta in infantile summer diarrhœa.	...	...	Yes.	Yes.	
9. *B. coli* (human).	Dejecta in cholera nostras.	Very.	...	...	Yes.	
10. ,,	,,	,,	...	...	,,	
11. ,,	,,	,,	...	...	,,	
12. ,,	,,	,,	Yes, in large doses.	...	...	

TABLE—*continued.*

Micro-organism experimented with.	Source.	Fatal to Guinea-pigs.	Fatal to Rabbits.	Non-fatal to Guinea-pigs.	Non-fatal to Rabbits.	Remarks.
13. *B. coli.*	Suspicious oysters.	...	...	Yes.	Yes.	
14. *B. coli.*	Suspicious waters.	...	...	Yes.	Yes.	
15. ,,		...	...	,,	,,	
16. ,,	,,	...	...	,,	,,	
17. ,,	,,	...	...	,,	,,	
18. ,,	,,	Very, in small doses.	...	...	Yes, toxic in large doses.	
19. *B. coli.*	Fæces.	...	...	Yes.	Yes	
20. ,,	,,	...	...	,,	,,	
21. ,,	,,	...	...	,,	,,	Very lively in its movements; abundant flagella.
22. *B. coli.*	Sewage.	...	...	Yes.	Yes.	
23. ,,	,,	...	...	,,	,,	
24. ,,	,,	...	...	,,	,,	
25. *B. coli.*	Suspicious milk.	...	...	Yes.	Yes.	
26. *B. coli.*	Human abscess.	Very.	Very.	...	...	
27. ,,	Human ovarian abscess.	Very, in small doses.	Very, in small doses.	...	...	
28. ,,	,,	Very.	...	...	Yes.	
29. ,,	,,	,,	...	...	,,	
30. ,,	Abscess in connection with gall bladder.	,,	...	...	,,	

CONSIDERATION OF THE RESULTS OF THE ABOVE TABLE OF EXPERIMENTS.

It will be seen from the above table that about half of the specimens experimented with were non-lethal in ordinary doses to what are roughly called the "common laboratory animals." Perhaps the want of toxic properties was in certain cases, at any rate as regards Nos. 2 and 3, and possibly in some others, due to attenuation or loss of virulence from repeated subcultivations, but even if a fair proportion of cases of non-fatal effects is allowed to this possible attenuation, there yet remain a considerable number of examples of the *B. coli*, which react to the tests considered to be characteristic of the species, but act like harmless water organisms when used to inoculate guinea-pigs and rabbits. This fact practically puts aside as valueless, as a test, inoculation experiments. Strangely enough this is especially to be remarked in connection with examples of the bacillus obtained from suspicious waters, from sewage, and from fæces, so that just where the test of inoculation might prove useful, it breaks down and must be given up. Of the specimens of the *B. coli* which proved to be lethal, a larger number were toxic to guinea-pigs than to rabbits, and, in fact, conformed rather to the *B. cavicida* type than to the *B. coli* as described by Escherich, which was fatal both to rabbits and guinea-pigs, or to the *B. neapolitanus* of Emmerich.

Possible explanations.—Of the possible explanations which might be suggested for the differences in the results of experiments we will put aside, however, first of all, as not applying to these experiments, the possibility that in some cases the animals had had a special immunity to the organism conferred upon them. This possible but somewhat improbable explanation might apply to a great discrepancy in results with one organism, supposing batches of animals were tested together with doses of the same culture of the organism, experimented with in proportion to their body weight. I have no record, however, of such a discrepancy appearing in my series of experiments. The three explanations suggested may be put shortly as follows:—

(*a*) That the *B. coli* in the experiments was in all cases of the same species, but that toxicity is a non-specific property, which is lost under certain circumstances. This is the usual explanation given to account for such differences as have been above described. It is a feasible explanation, and very possibly, as above mentioned, accounts for some of the differences between different organisms as regards toxicity. If, however, this explanation is a correct one, a serious difficulty arises from the fact that some specimens of the bacillus retain their toxicity when cultivated under exactly the same conditions as others which lose it.

(*b*) That the *B. coli* is of itself primarily a non-toxic organism, but that when cultivated under certain conditions the non-specific property of toxicity may be impressed upon it. This explanation might be considered a satisfying one, if it be true that all organisms which are isolated as and give the tests of the *B. coli* may be brought to similar degree of toxicity by the same artificial means. It is true that statements have been made that the virulence of any growth (of the *B. coli*) may be greatly augmented by passing the bacillus, by intrapleural inoculations, through a series of guinea-pigs or rabbits.[1] This has not, as far as I know, been done in every case, neither has a standard toxicity been by this means established. There is no doubt but that formerly the *B. coli* was looked upon as a harmless saprophyte inhabiting the intestines, and that now it is considered to be the cause of a number of different diseases. No doubt the suggestion of the possibility of adding toxicity would explain the fact that the *B. coli* isolated from inflammatory exudations are toxic, whereas those from water, sewage, and the like are much less so, if not harmless.

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[1] Thoinot and Masselin, "Bacteriology," English ed., 1899, p. 272.

(*c*) The third explanation is that grouped under the same head. we really have included different species of organisms, and that toxicity is the specific distinction the members of the at present named "colon group" of micro-organisms separating it into at least two species. I must confess that I am inclined rather to favour this view. It is really on all-fours with the observations of many investigators.[1] If this explanation is a correct one, then to include the *B. neapolitanus*, the *B. cavicida*, and the non-toxic *B. coli* under the same group is incorrect. Skilled observers have noted distinct differences in the effects upon animals of different microbes isolated from the fæces, intestinal mucus, and so on, which agree to a considerable extent in cultivation experiments, morphology, and the like. It seems to me to be quite as logical to separate them into different species, because of their markedly different effects upon animals, as to include them under one species, because they agree to a certain extent in morphology and growth upon various media. It is very difficult to believe, for example, that specimens Nos. 1, 26, and 27, a few drops of a broth culture of which introduced into the peritoneum will produce speedy death, is the same organism as that of which 5 c.c. of a broth culture perhaps produces no effect. I cannot help thinking too that some more recent results of several observers upon the result of inoculation experiments with the so-called *B. coli* lend support to the view that these organisms belong to different species. I mean that in some cases marked nervous symptoms are observed after inoculation with some specimens of supposed *B. coli*, certainly absent in the greater number of experiments. The animals suffer from atrophic paraplegia, accompanied by changes in the spinal cord, indicating anterior poliomyelitis. Such results have been recorded by Gilbert and Lion, and nearly similar experiences have been noted independently by Thoinot and Masselin.[2]

Relation of the B. coli to disease in the human subject.—Organisms which belong to the group of the colon bacillus have been found and isolated in connection with a considerable number of diseases in the human subject, as has been already indicated, of which we may enumerate peritonitis, enteritis, cholera nostras, epidemic diarrhœa, pleurisy, meningitis, and so forth. In the cases in which the bacillus may be cultivated from the blood, either during life or *immediately* after death, its causal relation to the disease is almost certain. But considering that the organism is, in at any rate typical cases, a true septicæmic bacillus its connection with affections such as diarrhœa is by no means clear, unless the blood is proved to contain it in great numbers. The same observation applies to its supposed effect in producing the symptoms of enteritis. The presence of *B. coli* in cholera stools, in inflammatory exudations, and so forth, in almost pure culture, might be explained on the supposition that in such excretions or exudations the bacillus has provided for it a particularly favourable nutrient medium for growth.

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[1] This is practically the conclusion come to by MM. Lesage and Macaigne (*Arch. de méd. expér. et d'anat path.*, Paris, 1892, p. 350). They distinguish two kinds of *B. coli*, *i.e. B. coli normal* and *B. coli pathologique*, according as the cultures have been isolated in normal cases or in cases of infectious diarrhœa; the former is not generally virulent, the latter has various degrees of toxicity.

[2] *Op. cit.*, p. 273.

XVIII.

Aus den „Research Laboratories of the Royal College of Physicians, London, and Royal College of Surgeons, England.“

Zur Pathologie des Coma diabeticum.

Von

Dr. Karl Grube, L. S. A. London.
Arzt in Bad Neuenahr.

(Mit 2 Curven.)

Die im folgenden zu beschreibenden Versuche wurden während des Winters 1899/1900 mit der Absicht ausgeführt, die von W. Sternberg¹) mitgeteilten Versuche einer Nachprüfung zu unterwerfen bezw. dieselben zu erweitern.

Das Verständnis des Coma diabeticum zu fördern und seine Ursachen aufzufinden, ist von dem grössten praktischen Interesse, da eine grosse Anzahl von Diabetikern im Coma stirbt, und da wir in Bezug auf die Behandlung desselben vollkommen machtlos sind. Wir können bis jetzt wenig mehr thun als zusehen, wenn bei einem Diabetiker Coma ausgebrochen ist, da wir von einer Behandlungsweise, die mehr als ganz vorübergehende Erfolge aufzuweisen hat, nichts wissen.

Wie schon gesagt wurde, sterben viele Diabetiker im Coma. Obwohl junge, von der schweren Form der Zuckerkrankheit befallene Personen einen höheren Procentsatz der Fälle abgeben, so muss doch betont werden, dass das Coma wie ein Damoklesschwert den Diabetikern droht.

Die erste Erwähnung, dass der Diabetes mit dem Auftreten von Coma enden kann, findet man in der noch heute lesenswerthen und lehrreichen Arbeit von Marsh aus dem Jahre 1854.²)

Das Coma diabeticum, so wie ich es in 13 Fällen beobachtet habe, beginnt in der Regel mit Verdauungsstörungen, bestehend in

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1) „Chemisches und Experimentelles zur Lehre vom Coma diabeticum“. Zeitschrift für klin. Medicin. 38. Bd. S. 65.

2) Dublin Quarterly Review. 1854. S. 1. „Observations an the treatment of diab. mcll.“

Appetitmangel, Uebelkeit, Erbrechen und Diarrhoe oder zuweilen auch hartnäckiger Verstopfung. Die Kranken nehmen rapide an Kräften ab. Der Harn enthält in der Regel neben Zucker grosse Mengen von Aceton, Acetessigsäure, β-Oxybuttersäure und Ammoniak. Die Acetonausscheidung kann gering sein oder auch ganz verschwinden, dagegen ist die Ausscheidung der Oxybuttersäure stets bedeutend, bis über 20 g in 24 Stunden. In der Regel ist auch Eiweiss vorhanden. Der Athem hat den bekannten an Chloroform erinnernden Geruch. Wenn diese Erscheinungen einige Tage gedauert haben, wird der Kranke schläfrig, benommen und allmählich comatös. Zuweilen tritt diese Benommenheit mit nachfolgendem Coma auch plötzlich ohne vorhergehende Verdauungsstörung auf, doch scheint dies seltener der Fall zu sein.

In manchen Fällen geht dem Stadium der Benommenheit ein solches grosser Erregung vorher; der Kranke ist unruhig, sein Gesicht ist geröthet, der Puls ist schnell und voll. Nach einigen Stunden folgt auf dieses Erregungsstadium der Zustand von Benommenheit. In zwei Fällen meiner Beobachtung traten während dieses Erregungsstadiums asthmatische Anfälle auf, welche in einem Falle so heftig waren, dass nur grosse Morphiuminjectionen Erleichterung gaben. In diesem Falle wiederholten dieselben sich in der Zeit von 2 bis 4 Uhr Nachmittags 3 mal, dann trat allmählich Benommenheit ein.

Einmal beobachtete ich das Auftreten heftiger Convulsionen in 4 mit circa ½ stündigen Pausen aufeinander folgenden Anfällen. In der Zeit zwischen den Anfällen war der Kranke bei Bewusstsein, wenn auch zerschlagen und ohne Erinnerung an das Vorhergegangene. Im Anschluss an den vierten Convulsionsanfall fiel er in Coma mit der typischen dyspnoischen Athmung. Es sei nebenbei bemerkt, dass dieser Kranke schon Tage lang auf grosse Dosen doppeltkohlensauren Natrons gesetzt war.

Das am meisten charakteristische Symptom für die Fälle von typischem diabetischem Coma ist die zuerst von Kussmaul beschriebene Athmung.1) Zuweilen beginnt diese Aenderung der Athmung schon einige Zeit vor dem Auftreten des Coma. So in einem Falle bei einer an schwerem Diabetes leidenden Dame. Dieselbe kam Nachmittags zu mir in die Sprechstunde mit geröthetem Gesicht, in grosser Erregung, mit trockner Zunge und ausgesprocher dyspnoischer Athmung. Sie glaubte sich erkältet zu haben. Am nächsten

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1) Deutsches Archiv für klin. Medicin. 1874. Bd. 14.

Morgen zwischen 5 und 6 Uhr trat Coma ein, obgleich sofort für energische Alkalibehandlung, gute Stuhlentleerung und Bettruhe gesorgt wurde.

Diese Form der Dyspnoe ist charakterisirt durch tiefe Inspirationen; die Luft wird mit grosser Kraft eingezogen, wobei der Thorax stark ausgedehnt wird. Die eintretende Luft verursacht bei jeder Inspiration ein ganz charakteristisches, einem tiefen Seufzer ähnliches Geräusch. Die Exspirationen sind kürzer als die Inspirationen und der Uebergang von Inspiration zur Exspiration hat oft etwas plötzliches; der Thorax scheint nach der gewaltsamen Ausdehnung bei der Inspiration mit einer Art von Ruck zu collabiren. Die Frequenz der Athemzüge kann vermindert und vermehrt sein. Häufiger sah ich das letztere. Wenn es dem Ende zugeht, werden die Athemzüge allmählich flacher und häufiger, um ganz kurz vor dem Ende ganz selten zu werden. In den wenigen Fällen, in denen ich darauf achtete, trat Stillstand der Athmung eher ein als der des Herzens.

Die Zunge ist im Coma trocken, hart und rauh wie ein Reibeisen. Die Körpertemperatur ist häufig subnormal. Der Puls und die Herzthätigkeit sind, ausser in den Fällen mit grosser Emaciation und Schwäche, kräftig und frequent und werden erst gegen Ende schwach.

Die Reflexe können, wie ich schon früher mittheilte,[1] und seitdem noch mehrmals beobachtete, bis kurz vor dem Ende erhalten bleiben. Die Pupillen sind meist mittelweit und reagiren auf Licht bis zum Ende.

Man hat dieses typische diabetische Coma auf die verschiedenste Weise zu erklären versucht.

I. Man hat es angesehen als eine Vergiftung durch Aceton, eine Ansicht, die bekanntlich zuerst von Petters[2] und Kaulich[3] vertreten wurde. Diese Theorie hat auch heute noch Anhänger. Ich halte sie aus folgenden Gründen für unrichtig: 1) Können Diabetiker jahrelang grosse Mengen von Aceton ausscheiden, ohne dass sich die geringsten Anzeichen von Coma zeigen; 2) hat man in einzelnen Fällen beobachtet, dass die Acetonurie verschwand, als Coma auftrat, was darauf hinzuweisen scheint, dass dasselbe nicht von dem Vorhandensein von Aceton abhängig ist; 3) haben Experimente dargethan, dass Aceton ausser in grossen Dosen nicht giftig ist, und dass die mit Aceton hervorgebrachten Erscheinungen nicht die des diabetischen Comas sind.

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[1] Neurolog. Centralblatt 1893. „Das Verhalten der Patellarreflexe beim Diabetes“.

[2] Prager Vierteljahrsschrift 1857. S. 81.

[3] ibid. 1860 S. 58.

II. Man hat das diabetische Coma als eine Form der Urämie ansprechen wollen. Das urämische Coma unterscheidet sich aber ganz ausgesprochen von dem typischen diabetischen Coma. Das erstere ist dazu stets von einer schweren Erkrankung der Nieren abhängig, während bei der diabetischen Form die Erkrankung der Nieren, wenn eine solche vorliegt, für das Coma nur von secundärer Bedeutung ist. Dass auch einmal bei einem Diabetiker Urämie auftreten kann, ist natürlich und ich habe das selbst beobachtet, aber dann ist die gleichzeitig bestehende Nierenerkrankung und nicht der Diabetes die Ursache.

III. Die Theorie, welche jetzt wohl die meisten Anhänger hat und besonders von Stadelmann, Naunyn, Magnus-Levy u. A. vertreten wird, führt das diabetische Coma auf eine vermehrte Säurebildung zurück. Stadelmann hat diese Theorie zuerst ausgesprochen, und ihm kommt auch das Verdienst zu, das Vorhandensein einer abnormen Säure zuerst gezeigt zu haben. Er glaubte, dass es sich um α-Krotonsäure handele, doch zeigten Minkowski und Külz, dass es sich um β-Oxybuttersäure handele. Walthers Experimente,[1]) der mit Chlorwasserstoff- und Phosphorsäure bei Kaninchen einen dem Coma ähnlichen Zustand hervorzurufen vermochte, scheinen ebenfalls im Sinne dieser Theorie zu sprechen.

Ausser der schon erwähnten β-Oxybuttersäure sind auch noch andere Säuren wie Acetessigsäure, Milchsäure und Fettsäuren im Harn gefunden und ebenfalls als für die Acidosis in Betracht kommend angesprochen worden, doch spielen nach Magnus-Levy[2]) neben der Oxybuttersäure andere Säuren quantitativ keine Rolle.

Die Auffassung des Coma diabeticum als einer Säurevergiftung hat zu der Behandlung mit grosssen Dosen von Alkali geführt, welche jedoch meines Wissens und nach meiner persönlichen Erfahrung überzeugende Resultate nicht aufzuweisen hat, ohne dass man das jedoch als gegen diese Theorie sprechend ansehen kann. Wenn der ganze Organismus durchsäuert ist, dann ist es an und für sich fraglich, ob es gelingen kann, genügende Mengen von Alkali dem Körper zuzuführen, um eine Neutralisation der Säure herbeizuführen. Das muss ich unbedingt zugestehen, dass ich in mehr als einem Falle von

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1) „Untersuchungen über die Wirkung der Säuren auf den thierischen Organismus.“ Archiv für exper. Pathol. u. Pharmakol. Bd. VII. S. 148.

2) „Die Oxybuttersäure und ihre Beziehungen zum Coma diab.“ Archiv für exper. Path. u. Pharmak. Bd. 42 S. 149.

Diabetes mit starker Acetessigsäureausscheidung und sonstigen Erscheinungen drohenden Comas eine wesentliche Besserung des Allgemeinbefindens und eine Verminderung der Acetessigsäure beobachtet habe, aber dieselbe hielt niemals lange Zeit an, obgleich das Alkali consequent weitergenommen wurde.

IV. Endlich ist noch einer Theorie zu gedenken, welche das diabetische Coma als den Ausdruck einer Vergiftung durch unbekannte Toxine ansieht. Der Hauptvertreter dieser Anschauung, welche an sich sehr viel Bestechendes hat, ist G. Klemperer.[1] Nach ihm ständen Coma und Ausscheidung bezw. Bildung von Oxybuttersäure etc. nicht im causalen Verhältniss zu einander, sondern sie wären coordinirte, durch eine gemeinschaftliche Ursache (Toxine) hervorgerufene Erscheinungen. Zweifellos lässt sich das Coma mit seinen Erscheinungen nach dieser Theorie am besten erklären.

Wir müssen nun etwas näher auf die Eingangs citirte Arbeit von W. Sternberg eingehen, in welcher derselbe auf Grund theoretischer Ausführungen und praktischer Versuche an Thieren zu der Ansicht kommt, dass in der β-Amidobuttersäure das unbekannte Toxin des diabetischen Comas gefunden sei.

Es war schon früher durch Experimente gezeigt worden, dass die beim Coma ausgeschiedene β-Oxybuttersäure, Acetessigsäure und Aceton nicht giftig sind, aber ihr regelmässiges Auftreten im Harn brachte Sternberg auf die Vermuthung, dass sie nur die Zersetzungsproducte einer Substanz seien, welche selbst giftig ist. Indem Sternberg sich die Symptome des diabetischen Comas vergegenwärtigte, kam er zu der Ueberzeugung, dass das hypothetische Gift zu den Amidokörpern gehören müsse, während chemische Erwägungen und die Thatsache, dass Binz und Mayer mit Buttersäure einen dem Coma ähnlichen Zustand hervorgerufen hatten, auf die Amidobuttersäure hinleiteten. Wenn nun angenommen wird, dass die im diabetischen Harn vorkommende β-Oxybuttersäure nur ein Zersetzungsproduct der Amidobuttersäure sei, so musste diese ebenfalls zur β-Reihe gehören, und der gesuchte Körper könnte demnach nur β-Amidobuttersäure sein.

Ich habe versucht, den Ideengang Sternbergs in aller Kürze wiederzugeben, ich muss aber den Leser ersuchen, die geistvolle Arbeit selbst zur Hand zu nehmen.

Nachdem theoretisch die β-Amidobuttersäure als das mögliche Toxin des diabetischen Comas festgestellt war, musste weiter der

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[1] Berliner Klinische Wochenschrift 1889. Nr. 40.

Versuch gemacht werden, mit derselben einen diesem Coma analogen Zustand hervorzurufen. Dies gelang Sternberg auch bis zu einem gewissen Grade. Er erzielte Coma, ferner eine Veränderung der Respiration, die etwa dem des diabetischen Comas ähnlich war, aber es gelang ihm nicht, das Vorhandensein von Aceton, Acetessigsäure und β-Oxybuttersäure im Harn der Thiere nachzuweisen.

Bei dem Interesse, welches die Frage hat, schien es mir der Mühe werth, die Versuche zu wiederholen. Vor allem schien es mir darauf anzukommen, dieselben in der Weise auszuführen, dass die eventuellen Zersetzungsproducte im Harn erschienen.

Die mir gestellten Fragen lauteten:

1. ob es möglich sei, Coma hervorzurufen,
2. ob es möglich sei, eine dem diabetischen Coma analoge Dyspnoe zu erzielen,
3. ob es möglich sei, die beim diabetischen Coma im Harn auftretenden Substanzen im Harn der mit β-Amidobuttersäure vergifteten Thiere nachzuweisen.

Die Experimente, 20 der Zahl, wurden mit einer Ausnahme (Kaninchen) an Katzen vorgenommen.

Die β-Amidobuttersäure bezog ich von Dr. Schuchardt in Görlitz. Sie stellte eine braune syrupartige Flüssigkeit dar, die ca. 90 Proc. Säure enthielt. Dieselbe wurde zum Versuch in destillirtem Wasser aufgelöst und durch Zusatz von etwas Ammoniak oder kohlensaurem Natron abgestumpft.

In der Mehrzahl der Experimente wurden Athmungs- und Blutdruckcurven genommen, um den Einfluss der Säure auf die Athmung und Herzthätigkeit festzustellen.

Die Methode war folgende: Das Thier wurde mit einer Mischung von Alkohol, Chloroform und Aether (Verhältniss 1 : 2 : 3) narcotisirt, auf den Rücken gelegt und aufgeschnallt. Hierauf wurde die linke Jugularvene freigelegt und durch eine Glaskanüle mit einer graduirten Burette verbunden, welche die Säure enthielt. Dann wurde die rechte Carotis freigelegt und mit dem Manometer verbunden. Eine Mareysche Trommel wurde über dem Abdomen dort fixirt, wo die Athembewegungen am deutlichsten waren. Die Bewegungen wurden durch einen Gummischlauch auf eine zweite Trommel übertragen, welche mit der Schreibvorrichtung in Verbindung stand. Nachdem der ganze Apparat functionirte und das Thier soweit aus der Narkose erwacht war, dass es auf Kneifen reagirte, wurde mit der Injection begonnen.

Versuch I/II: Kaninchen — 3120 g schwer.

11 h. 36 m. Injection von 1 g β-Amidobuttersäure aufgelöst in 18 ccm dest. Wasser in linke Ohrvene — Dauer der Injection 9 Minuten — ausser frequenter Herzthätigkeit nichts zu bemerken. Thier sitzt ruhig.

12 h. 11 m. 2. Injection von 1 g β-säure in 12 ccm Wasser. Thier sitzt ruhig.

12 h. 42 m. 3. Injection von 1 g β-säure in 12 ccm Wasser. Thier sitzt ruhig.

2 h. Das Thier scheint benommen, die Athmung ist tiefer und frequenter.

3 h. Thier noch stark benommen.

4 h. Thier wird lebhafter und läuft herum.

Versuch II. 1./II.: Katze — Gewicht 2800 g.

4,4 g β-säure in 22 ccm dest. Wasser aufgelöst.

3 h. 20 m. Injection von 10 ccm in linke Ingularis. Herz schlägt sofort kräftiger. Athmung frequenter und tiefer. Pupillen ad maximum dilatirt.

3 h. 35 m. Injection von 12 ccm der Lösung. Katze betäubt.

4 h. Katze wird unruhig, losgebunden kann sie nicht aufrecht stehen und fällt um, wenn sie auf die Füsse gesetzt wird.

4 h. 15 m. Katze kommt wieder zu sich, zeigt jedoch keine Neigung, sich zu bewegen.

5 h. Katze läuft durch das Zimmer.

Versuch III. 2./II.: Katze — Gewicht 3600 g.

3 h. 30 m. Injection von 2,8 g β-säure.

3 h. 40 m. „ „ 2,8 „ „ „

4 h. Pupillen ad maximum dilatirt; Thier stark betäubt. Respiration tief, 30 in der Minute, bei der Athmung wird der Thorax stark ausgedehnt, stärker als bei einer normal athmenden Katze.

4 h. 50 m. Thier erwacht aus der Betäubung, sitzt ruhig auf derselben Stelle. Herzaction sehr beschleunigt — Athmung normal.

5 h. 7 m. Katze läuft durch das Zimmer.

6 h. Katze hat sich anscheinend vollkommen erholt — getötet — der aus der Blase entnommene Harn giebt ausgesprochene Reaction mit Eisenchlorid.

Versuch IV. 3./II.: Katze — 3000 g.

Athmung vor Beginn der Injectionen 40 in der Minute.

12 h. Injection von 2 g β-Säure in 10 ccm Wasser.

12 h. 8 m. Injection von 2 g β-Säure in 10 ccm Wasser.

12 h. 20 m. Injection von 1 g β-Säure in 8 ccm Wasser.

12 h. 40 m. Injection von 1 g β-Säure in 10 ccm Wasser.

Nach der 2. Injection ist die Katze vollkommen betäubt, die Pupillen sind ad maximum dilatirt, die Athmung ist beschleunigt — ca. 50 in der Minute und die Insprationen sind etwas tiefer — ausgesprochen wird die Veränderung in der Respiration aber erst nach der 4. Injection, die Athmung wird dann verlangsamt, zuerst auf 30 in der Minute, später auf

24, die Inspirationen sind deutlich tiefer; costaler Typus der Athmung mehr ausgesprochen.

1 h. 30 m. Katze noch in voller Betäubung, wird getötet. Der der Blase entnommene Harn giebt ausgesprochene Reaction mit Fehling'scher Lösung und schwache Reaction mit Eisenchlorid.

Versuch V. 5./II.: Katze nach 5 tägigem Fasten,1) wobei das Gewicht von 2680 g auf 1980 g zurückgegangen war.

Athmung vor Beginn der Injectionen 42 in der Minute.
12 h. 18 m. Injection von 2 g β-Säure in 4,5 ccm Wasser.
12 h. 24 m. = = 2 g = = = 4,5 = =
12 h. 30 m. = = 2 g = = = 5 = =
12 h. 35 m. = = 2 g = = = 5 = =

Gleich nach der ersten Injection wird die Athmung tiefer, während der Blutdruck stark ansteigt. Die Pupillen sind stark dilatirt. Nach der 3. Injection wird die Athmung sehr oberflächlich und langsamer, während der Blutdruck nur während der Injection sinkt, um bald darnach wieder zur alten Höhe anzusteigen. Nach der 4. Injection wird die Athmung wieder tiefer und schneller. (Siehe Curve I.)

1 h. 20 m. Injection von 2 g Säure in 10 ccm Wasser. Nach dieser Injection wird die Respiration ganz oberflächlich und bleibt es bis zu dem um 1 h. 40 m. erfolgenden Tode, auch der Blutdruck wesentlich niedriger nach der Injection. Während des Experimentes liegt die Katze ruhig da, nur bewegt sie von Zeit zu Zeit den Schwanz, auch reagirt sie etwas auf starkes Kneifen.

In der Blase sehr wenig Harn; derselbe giebt mit Eisenchlorid eine schwache Reaction; keine Reaction mit Fehling.

Versuch VI. 6./II.: Katze — 2038 g.

Seit 10 Tagen nur mit Milch und Brot gefüttert.2)

1 h. Langsame Injection von 4,5 g Säure in 15 ccm destillirtem Wasser.

1 h. 10 m. Thier betäubt, reagirt aber noch auf Kneifen — Pupillen stark dilatirt. Kein wesentlicher Einfluss auf die Athmung zu constatiren.

1 h. 20 m. Schnelle Injectionen von 4,5 g Säure, die Athmung wird sofort oberflächlich, um ganz zu sistiren, der Blutdruck fällt sofort bedeutend und der Tod erfolgt nach einigen Secunden. Das Herz schlägt noch einige Zeit weiter, nachdem die Athmung schon aufgehört hatte.

In der Blase ca. 40 ccm Harn; derselbe enthält keine Acetessigsäure noch Aceton, gibt aber mit Fehling starke Reaction.

Versuch VII. 8./II.: Katze 3440 g.

12 h. 45 m. 2 g Säure injicirt.
12 h. 55 m. 3 g Säure injicirt.

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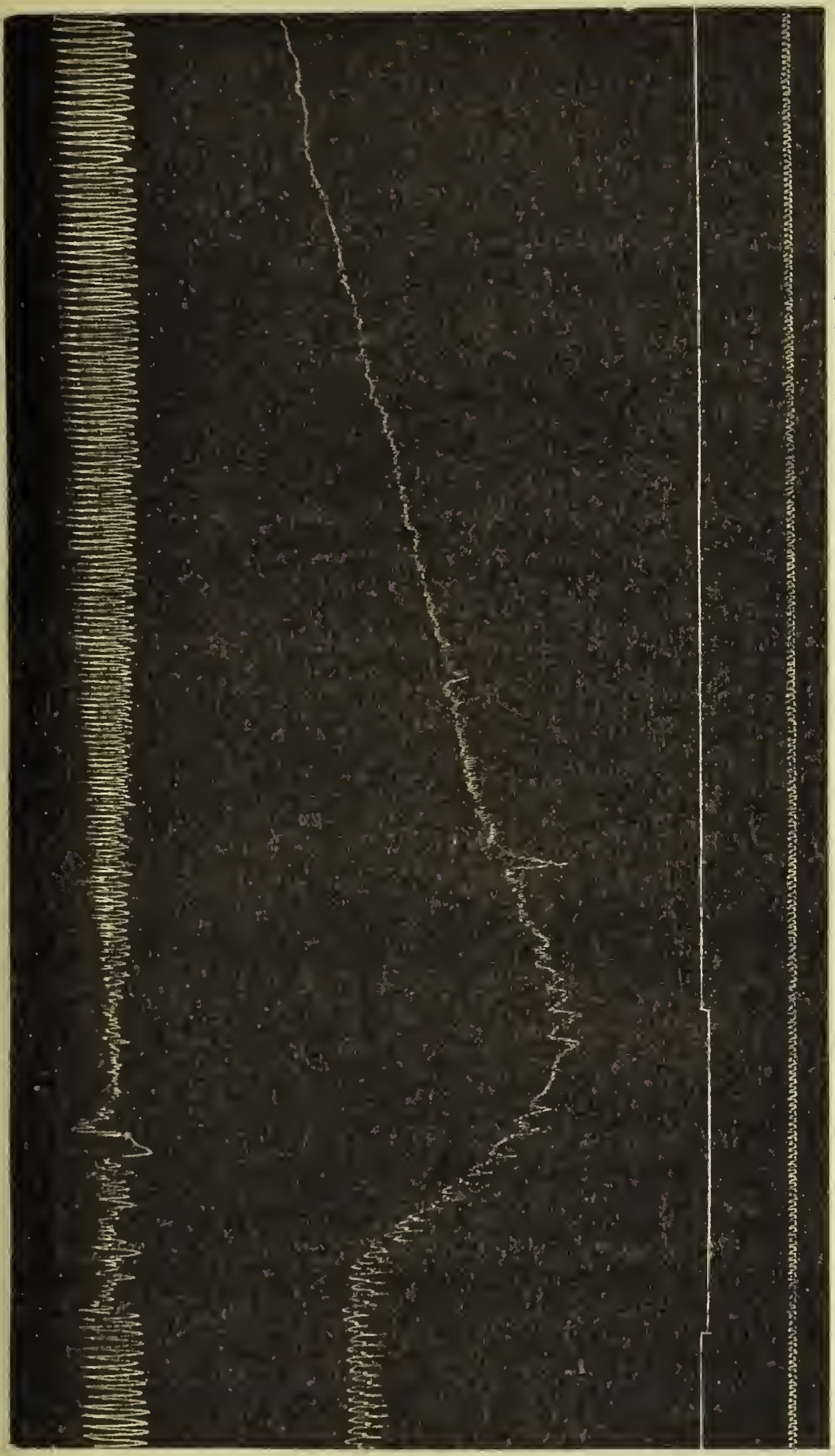
1) Die Katze hatte gehungert, damit nachgewiesen werden konnte, ob Zustand der Unternährung, wie er beim Diabetiker besteht, die Wirkung der Säure erhöhe. Das war nicht der Fall.

2) Alle andern Katzen wurden mit Pferdefleisch gefüttert.



C. I. Vertiefung der Athmung, speciell der Inspirationen und Ansteigen des Blutdrucks nach der Injection von 2 g β-Amidobuttersäure.

Pupillen dilatirt; Katze betäubt, reagirt auf Kneifen.

12 h. 57 m. 2 g Säure injicirt.

1 h. 20 m. Thier in tiefer Narkose, reagirt nicht mehr auf Kneifen. Athmung tief und schnell, ca. 40 in der Minute.

1 h. 45 m. Injection von 2 g Säure. Thier bleibt in tiefer Narkose, Athmung tief und ruhig.

3 h. Thier getödtet, bevor es erwacht ist. Blase voll.

Derselbe giebt folgende Reactionen:

1. starke Reaction mit Fehling,
2. deutliche Reaction mit Eisenchlorid,
3. das Destillat giebt deutliche Jodoformreaction (Lieben'sche Probe für Aceton).

Versuch VIII. 13./II.: Katze 2700 g.

10 g β-Säure werden in 30 ccm destillirtem Wasser aufgelöst und langsam, über $2^1/_2$ Stunden vertheilt, injicirt. Pupillen stark erweitert.[1] Katze fällt in comatösen Zustand, bleibt jedoch empfindlich für starkes Kneifen. Gleich im Anfange des Versuchs tritt eine Aenderung der Athmung ein, charakterisirt durch langes Inspirium und kürzeres Exspirium; Athemtypus stark costal. Der Blutdruck steigt im Anfang des Versuches an und hält sich bis zuletzt auf dieser Höhe; die Athmung wird gegen Ende langsamer und weniger tief. Nach $2^1/_2$ Stunden wird das Thier getötet. Blase voll Harn; derselbe giebt folgende Reactionen:

1. mit Fehling;
2. mit Eisenchlorid;
3. das Destillat die Lieben'sche Reaction.

Versuch IX. 14./II.: Katze 3570 g.

12 g β-Säure in 60 ccm destillirtem Wasser aufgelöst und langsam injicirt. Dauer des Versuches ca. 2 Stunden.

Katze vollständig comatös, reagirt auch auf Kneifen nicht. Pupillen ad maximum dilatirt. Athmung bis auf 18 verlangsamt — anfänglich 40 — sehr tiefe Inspirationen, gegen Ende des Versuches wird die Athmung oberflächlicher, bleibt aber langsam; der Blutdruck bleibt ziemlich auf gleicher Höhe. Der Tod erfolgte allmählich unter Nachlassen der Athmung.

In der Blase ziemlich viel Harn, derselbe giebt:

1. deutliche Reaction mit Fehling;
2. Gerhardt'sche Reaction;
3. Lieben'sche Reaction.

Versuch X. 15./II.: Katze 2700 g.

15 g β-Säure in 80 ccm Wasser aufgelöst und langsam injicirt. Dauer des Versuches über 2 Stunden.

Es wurde tiefes Coma erzielt. Pupillen anfangs stark dilatirt, später contrahirt. Athmung charakterisirt durch tiefe Inspirationen. Während des Versuches werden beide Vagi durchschnitten, um zu sehen, ob dieser Eingriff eine Aenderung der Respiration zur Folge haben würde; im

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1) Stets ein frühes Symptom der Injectionen.

ganzen blieb aber der Athmungstypus derselbe, nur wurde eine Verlangsamung der Athmung nach der Durchschneidung beobachtet. Nachdem das Experiment $1\frac{1}{2}$ Stunden gedauert hatte, trat ein kurzer heftiger Dyspnoeanfall ein und dann wurde die Athmung sehr oberflächlich, bis das Thier schliesslich an Athemlähmung zu Grunde ging. Der Blutdruck hielt sich bis gegen Ende hoch. Das Coma dauerte mit gleicher Tiefe während des Versuches an; es trat auf, nachdem 5 g der β-Säure injieirt waren; die oberflächliche Athmung trat auf, nachdem das Thier die ganze Menge von 15 g erhalten hatte.

Die Blase war gefüllt mit Harn; derselbe gab Fehling'sche und Gerhardt'sche Reaction und das Destillat gab die Lieben'sche Reaction.

Versuch XI. 20/II.: Katze — 3400 g.

Nachdem 8 ccm einer 15 g β-Säure in 80 ccm dest. Wassers enthaltenden Lösung langsam injicirt waren und die Athmung tiefer geworden war, trat plötzlich 4 Minuten nach Beendigung der Injection ein Stillstand der Athmung und der Tod ein.

Der in der Blase vorgefundene spärliche Harn gab nur leichte Reaction mit Fehling, sonst nichts.

Versuch XII. 20/II.: Katze — 3900 g.

60 ccm der Lösung vom Versuch XI. (15:80) werden langsam in der Zeit von 1 h 30 m bis 3 h injicirt. Es tritt Coma ein mit anfangs dilatirten, später contrahirten Pupillen.

Um 2 h 20 m wird die Harnblase durch Ausdrücken entleert und der Harn zur Untersuchung bei Seite gestellt, hierauf wird die Urethra mit Spencer Wells abgeklemmt, um das Entweichen von Harn zu verhindern; das Tier reagirt darauf garnicht.

Um 3 h wird die Katze vom Tisch genommen und in eine Ecke gelegt, wo sie ruhig liegen bleibt; 4 h 35 m wird sie getödtet.

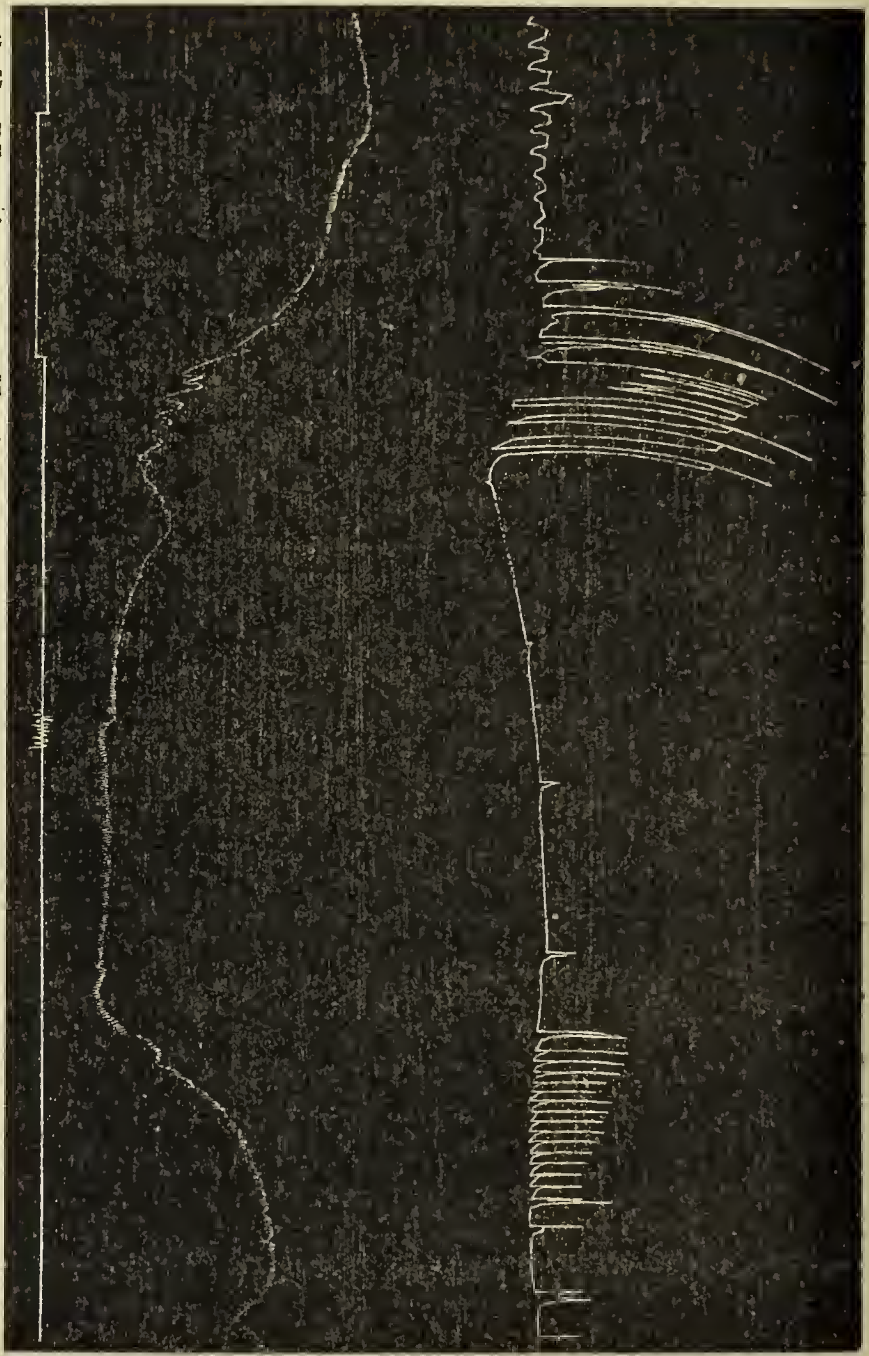
Harn a. giebt schwache Gerhardt'sche aber nicht die Lieben'sche Reaction. Harn b., der nach dem Tode der Katze aus der Blase entnommen wird, giebt ausgesprochene Gerhardt'sche und positive Lieben'sche Reaction.

Die Versuche XIII.—XVIII. ergeben dieselben Resultate wie die früheren, brauchen daher nicht im Einzelnen angeführt zu werden. Von grösserem Interesse dagegen sind die Versuche XIX. und XX.

Versuch XIX. 22/III.: Katze — 2750 g.

12 g β-Säure in 80 ccm dest. Wasser aufgelöst; gleichzeitig werden 100 ccm einer 2 proc. Natriumcarbonatlösung fertig gestellt. Nachdem 56,5 ccm der Lösung injicirt und Coma sowie die grosse Athmung erzielt worden waren, wurden um 3 h 45 m 5 ccm injicirt, worauf ein heftiger kurzer Dyspnoeanfall auftrat, die Athmung aber sonst noch tief blieb.

Um 3 h 50 m Injection von 4,5 ccm; diese hat ein Kleinerwerden der Athmung mit einzelnen sehr tiefen Inspirationen zur Folge, ganz kurz darauf wurden noch 8 ccm injicirt. Es traten nun zunächst eine Reihe sehr tiefer gewaltsamer Inspirationen auf, zwischen denen einzelne kürzere beobachtet wurden, dann wurden die Athemzüge kleiner und die Pausen zwischen den einzelnen Inspirationen sehr lang, während der Blutdruck stetig und nahezu bis zum Nullpunkt fiel. Als das Thier nahezu todt war, wurde schnell erst in den linken, dann in den rechten Oberschenkel 10 ccm der vorher fertig gestellten Natriumcarbonatlösung subcutan injicirt. Der Effect war ein nahezu plötzlicher, der Blutdruck stieg sogleich an und die Athmung wurde wieder tiefer und schneller. Nachdem noch-



C. II. Effect einer 2 proc. Na_2CO_3 lösung auf die Respiration und den Blutdruck nach Vergiftung mit β-Amidobuttersäure.

mals 10 ccm der Natronlösung subcutan gegeben worden waren, erholte sich die Athmung wieder vollkommen und erreichte nahezu wieder dieselbe Tiefe, welche sie gehabt hatte; auch der Blutdruck kehrte zur Norm zurück, das Coma dagegen blieb bestehen. Nach einiger Zeit (ca. 15 Minuten) wurden 6 ccm β-Säurelösung injicirt, die Athmung wurde sofort sehr oberflächlich und unregelmässig und trotz nochmaliger subcutaner Injection von 10 ccm der Natronlösung starb das Tier.

Der Harn gab starke Fehling'sche Reaction; die Reaction mit Eisenchlorid fiel schwach aus, desgl. die mit alkalischer Jodjodkaliumlösung.

Versuch XX.: Katze — 3000 g.

Ausser der linken Jugularis wird auch die linke Femoralvene freigelegt und durch Kanüle mit einer eine 2proc. Natriumcarbonatlösung enthaltenden Bürette verbunden. Die Injection der Amidosäure wird forcirt, bis Athemlähmung eintritt; hierauf werden 25 ccm der 2proc. Natronlösung in die Femoralvene injicirt, worauf sofort der Blutdruck ansteigt und die Athmung wieder kräftiger wird (Curve II.). Nach einiger Zeit (5 Minuten) werden nochmals 20 ccm Natronlösung injicirt, die Athmung kehrt darauf zur Norm zurück. Nachdem das Tier nach dreistündiger Dauer des Experimentes aus dem Coma erwacht, wird es getödtet. Der in der Blase erhaltene Harn giebt mit Fehling und Eisenchlorid positive Reactionen. Die Lieben'sche Probe mit dem Destillat fällt negativ aus.

Fassen wir die Resultate der Versuche zusammen, so ergiebt sich folgendes:

1. Coma oder ein comaähnlicher Zustand wurde in allen Fällen erzielt. Dasselbe vertiefte sich bei zunehmender Menge der angewandten Säure und war zuweilen absolut.

2. Gleich im Anfang der Injectionen trat Dilatation der Pupillen ein, welche längere Zeit anhielt, später aber in Contraction der Pupillen überging.

3. Die Athmung wurde deutlich beeinflusst. Bald nach dem Beginn der Injectionen wurden die Athemzüge tiefer und meist schneller; die Veränderung betraf die Inspirationen, welche tiefer und kräftiger wurden, besonders wenn grössere Mengen injicirt wurden. Zuweilen wurde die Athmung langsamer. In den Fällen, in denen das Thier mehrere Stunden lang im Coma gehalten wurde, wurde die Athmung gegen Ende oberflächlich, bis schliesslich Tod durch Stillstand der Athmung eintrat.

Der Typus der Athmung war ebenfalls verändert; derselbe wurde stärker costal, indem der Thorax bei der Inspiration kräftiger und ergiebiger ausgedehnt wurde, als es bei der Katze unter normalen Verhältnissen der Fall ist.

4. Bald nach der Injection wurde die Herzthätigkeit kräftiger

und beschleunigt; der Blutdruck zeigte während der Injection eine Abnahme, um dann anzusteigen. Er blieb während der Dauer des Versuches meist constant und höher als normal.

5. Harn wurde in 12 Fällen erhalten. Derselbe gab 9 mal die Fehling'sche, 10 mal die Gerhardt'sche und 6 mal die Lieben'sche Reaction.

Das Auftreten der Fehling'schen Reaction, die zuweilen sehr stark war, ist wohl auf die Operation zurückzuführen. Katzen reagiren bekanntlich auf operative Eingriffe sehr schnell mit einer Glykosurie.

Die Stärke der Gerhardt'schen Reaction war verschieden; sie nahm zu mit der Dauer des Versuches und der Menge der injicirten Säure.

Dasselbe gilt von der Lieben'schen Reaction; dieselbe fiel nur in den Versuchen von längerer Dauer positiv aus. In einem Falle, Versuch XII., gab der im Anfang des Experimentes gesammelte Harn die beiden letzten Reactionen nicht, wohl aber der am Ende des Versuches entnommene Urin.

Negativ fiel die Lieben'sche Reaction auch aus in den beiden letzten Versuchen, in denen das Alkali injicirt wurde.

Aus den mitgetheilten Experimenten wird man ersehen, dass die Versuche von Sternberg durch dieselben bestätigt werden, und dass die β-Amidobuttersäure einen Zustand hervorbringt, der mit dem diabetischen Coma grosse Aehnlichkeit hat. Darin unterscheiden sich meine Versuche von denen Sternberg's, dass es mir gelang, mehrere Male die Reaction für Aceton zu erhalten, und dass auch der Ausfall der Gerhardt'schen Reaction in meinen Versuchen anscheinend ausgesprochener war.

Weiter möchte ich aber nicht gehen, als nur die Aehnlichkeit der beiden Zustände, des diabetischen und des künstlich durch β-Amidobuttersäure hervorgerufenen Comas, festzustellen. Ob die chemischen Deductionen Sternberg's richtig sind, ob die β-Amidobuttersäure überhaupt im diabetischen Organismus gebildet wird, diese Fragen bleiben offen.

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ON THE NATURE OF THE SUGAR PRESENT IN NORMAL BLOOD, URINE AND MUSCLE.

By F. W. PAVY, M.D., LL.D., F.R.S. and R. L. SIAU.

(*From the Conjoint Laboratories of the Royal College of Physicians, London, and the Royal College of Surgeons, England.*)

BLOOD.

Magendie(1), Bernard(2), Schmidt(3), and others amongst former physiologists recognised a material in the blood which reduced the metallic oxides, as of copper, bismuth, and mercury, on boiling in the presence of an alkali and which underwent alcoholic fermentation in contact with yeast. They thence concluded that sugar in the form of glucose was present.

By Fischer's discovery of the osazones arising from the combination of carbohydrates with phenyl-hydrazine a method was provided for reliably distinguishing one sugar from another in a manner that could not be accomplished before. von Jaksch(4), Külz(5), Pickhardt(6) and Miura(7) applied the process to the product obtainable from blood and obtained a crystalline osazone which agreed in form with glucosazone. The last three observers also determined the melting point of the osazone and found it to stand at 204° or 205° C. which is the melting point of the glucose compound. Hanriot(8) operating upon 30 litres of horse's blood obtained after repeated purification about 6 grams of a substance corresponding in optical activity and in reducing power with dextrose. By treating a portion of the substance with anhydrous chloral he prepared the so-called parachloralose(9) with the characteristic melting point of 227° C.

Up to the present time it may be said that dextrose has been generally believed to constitute the form of sugar existing in blood, and one of us writing in 1894(10) adopted this view. His experimental results were not in absolutely strict accord with it but the divergence was not sufficient to lead him to consider himself justified in differing from it.

His experiments consisted in subjecting the product obtained by alcoholic extraction from blood to the inverting action of sulphuric acid. Sulphate of soda was employed preparatory to titration to precipitate the colouring matters, fats, etc., which are present with the sugar. It was known that the hydrolysing power of the sulphuric acid was much interfered with by the sulphate of soda, and a temperature of 150° C. obtained by the use of an autoclave was employed to meet the difficulty. It was felt that at such a temperature danger was incurred of some sugar destruction but no other way out of the difficulty presented itself till the hydrated alumina process of depuration was adopted(11). By this process a clear pale liquid is yielded which contains nothing to interfere with the hydrolysing action of acid. Hydrolysis is now effected with hydrochloric acid under an inverted condenser.

Since the employment of this method the results have shown that the reducing power is to a marked extent greater after than before inversion. The following examples may be adduced to illustrate the difference observable.

The figures in the first two columns represent the CuO reducing power expressed as dextrose per 1000 of blood before and after hydrolysis. Those in the column headed *K* represent the percentage relation of the CuO reducing power observed before hydrolysis to that found afterwards and it is seen that they depart widely from 100, which, with a slight allowance for errors of observation, would be the value if dextrose were the only carbohydrate present.

Source of blood	Sugar $^0/_{00}$ Before hydrolysis	After hydrolysis	*K*
Dog	0·860	1·300	66
Cat	1·026	1·409	72
Rabbit	1·116	1·700	65
Horse	1·160	1·550	75

Besides these examples attention may be directed to the results already published in this *Journal*(12). In these *K* has a mean value of 75.

Glycogen cannot possibly be the source of the increased reducing power observed, as the strength of the alcohol (methylated spirit) used in extracting the blood is too great to allow of any being dissolved.

In view of these results, showing that an increase in CuO reducing power is produced by the hydrolysing influence of a mineral acid, and suggesting the presence of a carbohydrate besides dextrose, the assistance of the phenyl-hydrazine reaction was sought to throw light upon the matter.

As a considerable quantity of blood was required for working upon it was felt that the horse would supply the most suitable source and through the assistance of Professor Woodhead an opportunity was presented for obtaining what was wanted.

A horse in a healthy condition which was about to be used in the preparation of anti-diphtheritic serum but which had as yet received no injection of toxine was employed. From the first bleeding from the jugular 2 litres of blood were collected in about 8 litres of methylated spirit and taken for analysis. The spirit was kept in motion to secure the formation of a fine coagulum. By this collection direct into alcohol no opportunity was afforded for the occurrence of any post mortem change.

Another portion of blood was taken for the quantitative determination of the sugar by the ordinary method, sodium fluoride being added to prevent change during transit to the laboratory. The result obtained is represented in the table above.

After 24 hours the alcohol was strained off from the precipitate which was strongly pressed and after being ground down was again extracted with alcohol for 24 hours at the room temperature. The mixed extracts were distilled down at 40° C. under reduced pressure to about 2 litres and divided into two approximately equal portions *A* and *B* in order that a portion might be subjected to examination in relation to the question of jecorin.

Portion *A* was examined in the ordinary way for sugar. It was concentrated on a boiling water bath to 100 c.c. and was then boiled briskly with alumina and filtered. The filtrate after being treated with lead acetate and subacetate and the excess of lead removed by sulphuretted hydrogen was further concentrated to 50 c.c.

Of this liquid 10 c.c. were diluted and titrated. The *K* was found to be 75.

The rest of the liquid was mixed with 0·5 grams of phenyl-hydrazine dissolved in 5 c.c. of 30 per cent. acetic acid and heated for 1½ hours on the water bath. A considerable quantity of crystals (α) had then separated and were rapidly filtered off. The filtrate was returned to the water bath and allowed to cool down very slowly when a further quantity of crystals (β) separated.

Crystals (α) had the form of glucosazone and after recrystallisation from acetone melted at 203° C.

Crystals (β) were collected, washed with cold water and suspended in hot water for ¼ hour when all but a small portion (γ) dissolved.

Crystals (γ) collected and washed with hot water and afterwards with acetone had the form of glucosazone but melted at 193° C. The amount was too small to recrystallise.

The hot filtrate from crystals (γ) was cooled very slowly when a bright yellow crystalline precipitate separated. The precipitate which consisted of spherical aggregates of needle crystals was collected and recrystallised several times from hot water when the crystalline form remained unaltered but when dried on porcelain the pale yellow crystals aggregated to an orange-brown mass. The melting point of this fraction was 157° C.

Proof was here afforded of the presence of an osazone different from glucosazone.

Portion *B* of the original alcoholic extract was further concentrated in vacuo at 37° C. After first being extracted with wet ether to take up jecorin if present, it was treated in the same manner as portion *A*; and again, besides glucosazone, another osazone was obtained which crystallised from hot water in spherical aggregates of needles melting at 158° C.

As the alcoholic extract of the blood had not at any time been heated above 40° C. the ether extract should, according to the statements of Henriques, Manasse and others(13), contain as jecorin the greater part of the sugar present in the blood. It was therefore washed with a little water and evaporated down. The residue after being taken up in water had no cupric oxide reducing power either before or after hydrolysis with hydrochloric acid, showing that it contained neither jecorin nor sugar. The water washings of the ether were concentrated and found to reduce cupric oxide very slightly. This was considered to be due to a trace of sugar which had been dissolved by the wet ether and was not followed further.

The above results show that blood contains, besides the sugar yielding with phenyl-hydrazine glucosazone, another sugar giving an osazone freely soluble in hot water and crystallising in spherical aggregates of needles melting at 157–8° C.

Owing to the marked solubility of this osazone in mother liquor containing phenyl-hydrazine and the products of its decomposition it has been found to be of considerable importance that the excess of phenyl-hydrazine used should be slight. In a dilute solution with much phenyl-hydrazine present it may easily fail to crystallise out and may thus escape recognition. In the case of glucosazone no such difficulty arises.

URINE.

Brücke pointed out how by certain processes the sugar could be separated from large quantities of healthy urine and obtained in a state to give all the ordinary reactions. The process was put into practice a number of years ago by one of us (Pavy [13 a]) and the presence of sugar demonstrated by various tests.

Since Fischer's discovery of the osazones producible with phenylhydrazine no room for doubt has been left as regards the presence of sugar in normal urine. Of the earlier authorities who showed that a sugar-osazone is susceptible of being obtained from normal urine mention may be made of Roos (14) (1891), Salkowski (15) (1892), Baisch (16) (1894), and Pavy (10) (1894).

Baisch (16) conducted a careful and extended enquiry into the question of the nature of the carbohydrate that is present in urine and found that it was not limited to dextrose. By the agency of benzoyl chloride he extracted the carbohydrate matter in the form of esters. Saponifying these esters with sodium ethoxide, he obtained a product which was found to contain "animal gum" and which yielded two osazones, one agreeing in its characters with glucosazone, and the other not. The second osazone differed from glucosazone not only in crystalline form but also in being soluble in hot water and in having a melting point of about 153° C. Baisch regarded it as corresponding with Fischer's (17) isomaltosazone. The parent sugar of the osazone was not fermentable by yeast.

Lemaire (18) repeated and confirmed Baisch's observations and agreed with him in identifying the second osazone with that of Fischer's isomaltose. Thus normal urine has been recognised as containing not only glucose but likewise a sugar of the maltose type. Recently Rosin (19), and Alfthan (20) who worked under his guidance, have shown that diabetic urines contain in addition to dextrose increased quantities of "animal gum" and of a substance which they say is unfermentable and yields an osazone corresponding to that described by Baisch and Lemaire, and following them they call it "isomaltose."

We have on many occasions prepared and fractionated osazones from healthy urines with like results to those described above. We have also similarly dealt with diabetic urines and readily obtained the "isomaltosazone" product, particularly in cases where only a limited quantity of sugar was being eliminated in association with a fair amount of carbohydrate ingested—that is in mild cases of glycosuria.

In accord with the evidence afforded by this line of investigation it is noticeable that the urines in question acquire an increased cupric oxide reducing power by subjection to the hydrolysing influence of boiling with an acid.

As illustrations of this condition the following cases may be cited.

I. There was no indication of the subject being otherwise than in good health and he was partaking of an ordinary mixed diet when the specimen of his urine examined by us was passed. The CuO reducing power was raised by boiling with acid from 4·03 to 6·11 $^{0}/_{00}$ expressed as glucose. Treated with phenyl-hydrazine, glucosazone and "isomaltosazone" were obtained.

II. Here the CuO reducing power of the urine was increased by treatment with HCl from 4·31 to 6·05 $^{0}/_{00}$. Glucosazone and "isomaltosazone" were obtained.

III. A case of mild glycosuria. The CuO reducing power of the urine examined was equivalent to 13·7 $^{0}/_{00}$ of glucose before and 20·8 $^{0}/_{00}$ after treatment with HCl, giving $K = 66$. The dextrose indicated by the polariscope was 11·5 $^{0}/_{00}$. Examined for pentoses with phloroglucin and HCl a very slight red coloration was obtained.

A portion of the urine was treated with yeast and allowed to stand for 3 days at 20° C. The CuO reducing power in terms of glucose was then found to be expressive of 2·7 $^{0}/_{00}$ before and 7·2 $^{0}/_{00}$ after treatment with acid, $K = 38$. The fermented liquid showed levo-rotation equal to the dextro-rotation corresponding with 1 $^{0}/_{00}$ of dextrose. It gave no coloration with phloroglucin and HCl. On treatment with phenyl-hydrazine, after removal of the alcohol produced by fermentation, a trace of glucosazone and a somewhat larger amount of "isomaltosazone" were obtained.

IV. The subject of this case occasionally excreted cupric oxide reducing urine. In the specimen of urine examined there was apparently only a trace of glucose present but a considerable quantity of "isomaltose."

250 c.c. of the urine which gave a slight but definite reaction with Fehling's solution was boiled with mercuric chloride and sodium acetate and, after being freed from mercury by zinc dust, treated with phenyl-hydrazine. No osazone separated till the liquid cooled when a

plentiful crop of crystals formed. This was almost entirely soluble in 200 c.c. of hot water from which it crystallised in pale yellow clusters of fine needles. After being recrystallised from its solution in water at 70° C. the purified osazone melted at 152—4° C. In drying on porcelain the pale yellow osazone ran together into a red-brown mass.

Pentoses were sought for in the urine with the phloroglucin and HCl test and a negative result obtained.

MUSCLE.

The diverse opinions that were expressed in former times as to the presence of sugar in muscle need not be referred to here. We will pass direct to those statements entitled to receive attention by reason of the greater reliability of the methods adopted.

Panormoff (21) extracted from mammalian and fish muscle a sugar the osazone of which he identified as glucosazone from its physical characters, melting point, and elementary composition. He also prepared from it pentabenzoyl-glucose with the normal melting point but he was unable to obtain any fermentation of his sugar solutions, a failure which he ascribes to their great dilution. Besides the osazone referred to above Panormoff further separated an osazone with a melting point of 152–3° C. by extracting his crude osazone with boiling water and collecting the crystals that formed. He makes no suggestion as to the parent sugar of this osazone but states that it cannot be maltose.

We have long known that the CuO reducing power of an alcoholic extract of muscle becomes considerably increased by subjection to the hydrolysing influence of an acid, and upon this ground it was suggested by one of us in 1881 (22) that maltose might be present. In 1894 (10) the author referred to demonstrated by means of photo-micrographs that by treatment of muscle extracts with phenyl-hydrazine glucosazone crystals were obtainable.

We have since investigated further the question of the osazones obtainable from aqueous and alcoholic extracts of mammalian muscle. There has been no difficulty in obtaining evidence of glucosazone which on account of its very sparing solubility more readily crystallises out than other osazones. Even with it, however, a certain amount of impediment to crystallisation has been found to be offered by the impurities present. We therefore determined to have recourse to the benzoyl-chloride process, as adopted by Baisch for the urine, for separating the carbohydrate matter from aqueous extracts of muscle. The esters were afterwards saponified with sodium ethoxide in the usual way. By means of this method "isomaltosazone" crystals were obtained and confirmation was supplied of the results of Panormoff.

CONCLUSIONS.

From what has preceded it is seen that it is not correct to speak of the sugar of normal blood as consisting only of dextrose. In reality it stands in accord with that found in normal urine and in muscle. All stand upon the same footing in yielding evidence of the presence of dextrose and of another sugar which gives an osazone that is soluble in hot water, and has a crystalline form quite distinct from that of glucosazone and a much lower melting point, namely about 153–5° C.

By Baisch and Lemaire this other sugar has been regarded as identical with Fischer's isomaltose.

Fischer (17) who was the first to make use of the term "isomaltose" applied it to a product which he obtained by acting upon dextrose with fuming hydrochloric acid in the cold. This product, which however he never actually isolated, was found by him to give an osazone melting at about 153° C., soluble in hot water, and having the composition of the osazone of a disaccharid.

A similar osazone was prepared by Scheibler and Mittelmeier (23) from the gallisin found by Schmidt and Cobenzl (24) in the unfermentable residue of commercial glucose.

C. J. Lintner (25) claimed to have found "isomaltose" in beer residue and, later (26), among the products of the limited action of diastase on starch, but it has been shown by Ling and Baker (27), Brown and Morris (28), and more recently Prior and Wiegmann (29), that the osazone on which Lintner's evidence rests is maltosazone altered in form and melting point by being crystallised from the solution of the uncrystallisable osazone of a malto-dextrin.

"Isomaltosazone" was prepared by Röhmann and Spitzer (30) from dog's liver and by Külz and Vogel (31) from the product of the action of the amylolytic enzyme of saliva and pancreas on glycogen. The product from these sources is probably similar to Lintner's "isomaltosazone."

Whatever may be the precise nature of the body to which the term "isomaltose" has been applied, it is evident that it is something distinct from glucose. It therefore follows from the results that have been obtained that glucose is not the only sugar present in blood. The same applies to urine and muscle and inferentially probably also to the other structures of the body.

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THE COMPOSITION AND ACTION OF ORCHITIC EXTRACTS. By WALTER E. DIXON, M.D., Lond. (Twenty-five Figures in Text.)

(*From the Pharmacological Laboratory, Cambridge, and the Research Laboratories, R.C.P. and R.C.S. London.*)

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HISTORICAL.

It is now about eleven years since Brown-Séquard(1) announced before the Collège de France the effect of certain experiments which he had performed upon himself, viz., the subcutaneous injection of the expressed juice of the testes of dogs and guinea-pigs. As a result of these injections he stated that he felt ten years younger, his capacity for both mental and physical work was increased, and that efficient peristaltic movements which had been absent for years returned. He did not consider that the increase of power could be explained on the assumption of simple stimulation as the effect lasted too long, but believed it to be more likely dymogenic in nature. He used a glycerine and water extract which was filtered and sterilized.

Variot(2) experimented with three old men, using the testes from the rabbit and the guinea-pig, and recorded the same "rejuvenating effect," the increased appetite for physical work and the stimulation of peristalsis. Pasteur, Bogroff⁽³⁾, Boch, Zenetz and a host of other observers confirmed the extraordinary statements of the original investigator, whilst many others were found to disparage his results⁽⁴⁾. Hénocque⁽⁵⁾, from a series of experiments on phthisical patients found the hæmoglobin was permanently increased and hence believed that orchitic injections had a beneficial effect on the blood. At the present time clinical evidence as to this method of medication is conflicting, possibly the variety and mode of preparation of the extract used, as well as the difficulties in the method of administration, being responsible for the want of uniformity in results: some clinicians have exactly followed Brown-Séquard's method, others have sterilized the fluid by various doubtful means, whilst still a third class have administered the gland by the mouth.

With regard to the active principle, more than one substance has been suggested. Luton found injections of sodium sulphate and phosphate as efficacious as the testicular extract, whilst Poehl, Bubis, and Rotchinum considered that spermine injections corresponded to the injection of Brown-Séquard's fluid. Brown-Séquard⁽⁶⁾ denied that either spermine or the Charcot-Naumann crystals were the active constituent of orchitic extract.

The scientific literature on the subject is extremely meagre. Zoth⁽⁷⁾ performed a series of experiments with the ergograph of Mosso, and found that injections of orchitic substances produced a marked increase in the power of muscle for performing work; Pregl⁽⁸⁾ with the same instrument came to somewhat similar conclusions. It is an open question, however, as to what reliance can be placed on this type of experiment in view of the misleading results which have been obtained with it in other directions.

Oliver⁽⁹⁾ observed moderate constriction of vessels in the frog's mesentery as the result of direct application of testicular extract.

Hedbom⁽¹⁰⁾ stated that orchitic extract produced increased force and rapidity of cardiac contractions, lasting a considerable time; the experiments were performed on the isolated cat's heart. Cleghorn⁽¹¹⁾ recorded the contractions of the apical portion of the ventricle of the dog's heart which was kept beating by feeding it with blood according to Porter's method. When perfused with orchitic extract, he observed an increased force of apical contraction, but without any change in tonus or rhythm. Larger doses produced a marked diminution in tonus and some quickening of the beat.

METHOD. ANIMALS. ANÆSTHESIA.

The blood-pressure was taken in every case from the right carotid with a mercurial manometer. Injections were made into the jugular vein unless otherwise stated. The respiratory movements were registered by strapping a tambour to the animal's chest, which was connected by tubing with a recording tambour: the up stroke registered inspiration. Air oncometers were employed for studying the changes in volume of organs; these communicated usually with a piston recorder. The Thoma-Zeiss instrument was used for counting leucocytes, and a solution of one-third per cent. acetic acid containing a trace of methyl violet was employed as the diluting fluid. The staining reagent for differential counts was one of the eosin methylene blue mixtures, and the counting was performed with a $\frac{1}{12}$ oil immersion and a No. 5 eye-piece.

The animals employed were cats, dogs, goats, and rabbits. Cats were the chief animals used, were full grown of either sex and weighed between 3 and $3\frac{1}{2}$ kilos.: it should be understood that the experiments in all cases refer to these animals unless it is otherwise stated. The carnivorous animals were anæsthetised with A.C.E. mixture and the herbivorous with ether. Morphia was also usually employed in the case of dogs, but never with the other animals. Urethane was administered as an anæsthetic in a few cases which are described in the text. It was given by injection into a vein, after the animal had been initially anæsthetised with A.C.E., but in one series of experiments it was administered by the mouth and no other anæsthetic was employed.

PREPARATION OF THE EXTRACT.

The testes of full-grown rats or guinea-pigs were employed usually for the purpose of this investigation. The animals were killed by a single blow on the head and the testes were immediately dissected out, washed as free from blood as possible, sliced into pieces and macerated for half-an-hour with physiological saline, using about 5 c.c. for each testis. This emulsion was strained through muslin and filtered through an ordinary paper, and the solution thus obtained was ready for use. The epididymis was included in the extract, but in some cases extracts of epididymis were prepared separately and will be referred to later. No advantage or alteration in the physiological action of the extract was observed as the result of a long extraction in the cold (24 to 48 hours).

Testes from other animals, dog, cat, goat and ram, were employed in a few cases, and the results obtained led to the conviction that the active constituents were the same in all. The testes of the rat and guinea-pig gave the most satisfactory results as, on account of their paucity in connective tissue and softness in structure, it was easier to obtain a satisfactory extract.

An attempt was made to obtain an extract having a constant strength. A quantity of ram's testis was cut up and thoroughly dried at 38° C.[1]; this was ground in a mortar to as fine a powder as possible, and a known weight extracted with normal saline for two or three hours: the extract was strained and filtered, and was then ready for use.

The results obtained with this solution were not, however, quite the same as those obtained with the fresh extract, because animal extracts dried in this manner do not readily allow their proteids to enter into solution. It was found that although an active solution of constant strength was obtained, yet it did not represent the same relative percentage of constituents as in the fresh extract; nevertheless by using sufficient quantities all the essential effects were produced. Fresh testicular extract will only keep a few hours at ordinary room temperature, and hence it is necessary to prepare a fresh extract for each series of experiments, and with each extract to make a control experiment to gain an idea of the activity of the solution. Different extracts show considerable variability in strength, the nature of the animal, age, condition, season, etc., no doubt being largely responsible for these differences.

EFFECT ON BLOOD-PRESSURE.

Cat. The injection of the extract ($\frac{1}{2}$ to $\frac{3}{4}$ of a rat's testis) into the circulation of a cat produces an immediate, though slight fall in blood-pressure followed by a quick recovery: for convenience in subsequent description this will be known as the fall "A." "A" is succeeded in a time varying from 30 to 50 seconds, by a second fall of pressure "B" which is associated with cardiac inhibition. Recovery is gradual, and complete in a few minutes.

Each subsequent injection produces a diminished effect. After a lethal injection (about 1½ testes) the pressure at "B" falls to nearly zero, and the heart beats slower and slower and ultimately stops; respiration ceases permanently at the outset of the cardiac inhibition. Death is always produced as a result of cardiac and respiratory failure, and intravascular coagulation of the blood is never observed.

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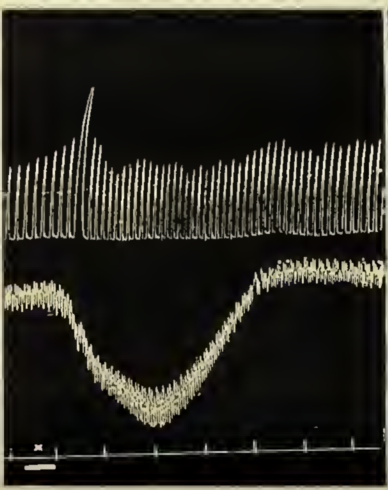
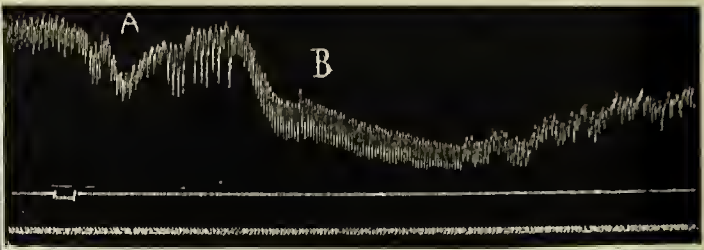
[1] Kindly provided by Messrs Burroughs and Wellcome.





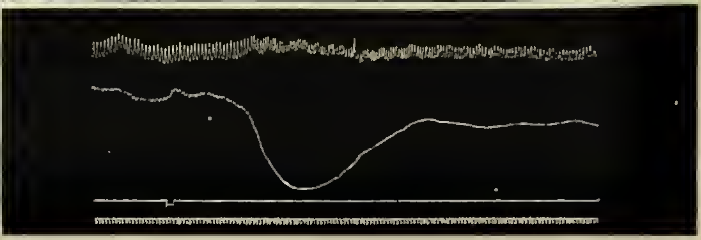


Fig. 2. Fig. 1 (upper figure). Fig 3. (lower figure).

Fig. 1. *Cat.* Injection of an extract composed of half a guinea-pig's testis. The fall "B" is associated with cardiac inhibition and cessation of respiration. Time marker = seconds.

Fig. 2. *Dog.* Morphia. Upper tracing represents respiration, the up stroke being inspiration. Injection of dried orchitic extract 0·5 grm. Time marker = 10 seconds.

Fig. 3. *Rabbit.* Respiration and carotid pressure. Up stroke represents inspiration. 1 c.c. orchitic extract injected (5 c.c. = 1 rat's testis). Time marker = seconds.

Dog. A single fall of pressure only is noticed. It begins after a very short latent period, and is accompanied by some cardiac slowing. This effect corresponds to the "A" effect in the cat as is shown by the short latent period.

The body producing the "B" effect in the cat is much less active in the dog, and after a small injection as shown in Fig. 2, the "A" effect alone is seen. Larger injections produce a more considerable fall of pressure with a slow recovery.

The **rabbit** is very easily affected and small injections only must be given. In this animal as in the cat there is an immediate small fall in blood-pressure, followed about half a minute from the time of injection by a more considerable fall associated with cardiac inhibition. Recovery is at first rapid, but the normal pressure is not reached for some minutes. In Fig. 3 the "B" fall only is clearly distinguishable, but the two are clearly shown in Fig. 6 after a much bigger injection.

The **goat** behaves differently to other animals in its reaction to testicular extract. Relatively small injections produce an initial ascent of pressure corresponding to "A," followed by a quick drop to normal; this is succeeded after a latent period of 40 seconds, by a second and more considerable rise of pressure lasting some two or three minutes.

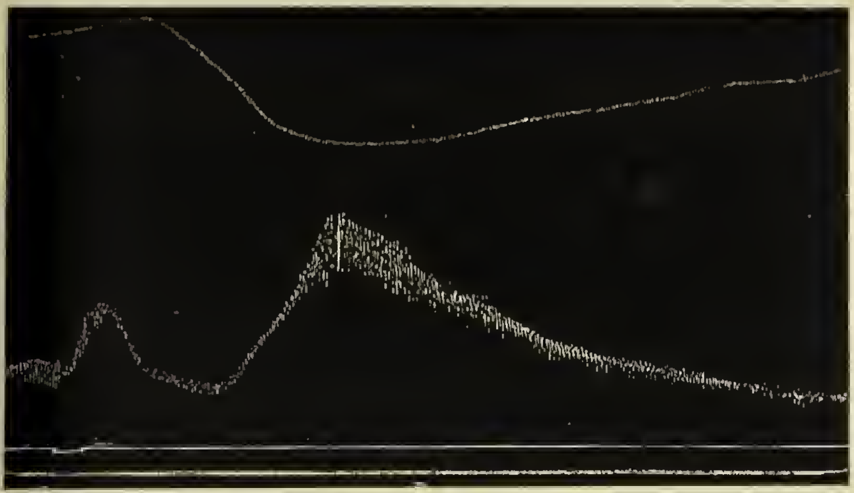


Fig. 4. *Goat.* Upper tracing represents the volume changes of one testis. Lower = carotid pressure. Time marker = seconds.

It should be noted that the amount injected into the various animals was not proportional to their respective weights; thus the amount injected into the cat was in every case, relatively to the dog and goat, largest, and in the goat much the smallest.

EFFECT ON THE HEART.

The direct application of the extract to the frog's heart, recording by the suspension method, produces no effect: when the extract is perfused through the heart there is a gradual diminution in the force of the beat, and this effect is to some extent antagonised by the use of atropine.

For studying the effect on mammalia, the heart of the cat was chosen. In one series of experiments the chest was opened, artificial respiration employed, and hooks fixed in the epicardium of the right auricle and left ventricle. Threads connected these by means of pulleys to suitably weighted levers free to record vertically.

Two distinct effects can be observed after an injection of orchitic extract, one corresponding to the "A," and one to the "B," fall in blood-pressure. During the first effect the rhythm of the heart is slowed a little, and the excursion of the auricular lever extends slightly further both upwards and downwards, indicating a more efficient systole and diastole. During the same period, the ventricle does not behave in the same way: relaxation of the muscle is more complete and the force of contraction is diminished. This is the "A" effect.

Recovery from this effect is hardly complete when the second effect begins. The auricular systole becomes gradually weaker in strength, diastole being much less affected; at the same time the rhythm of the heart becomes much slower.

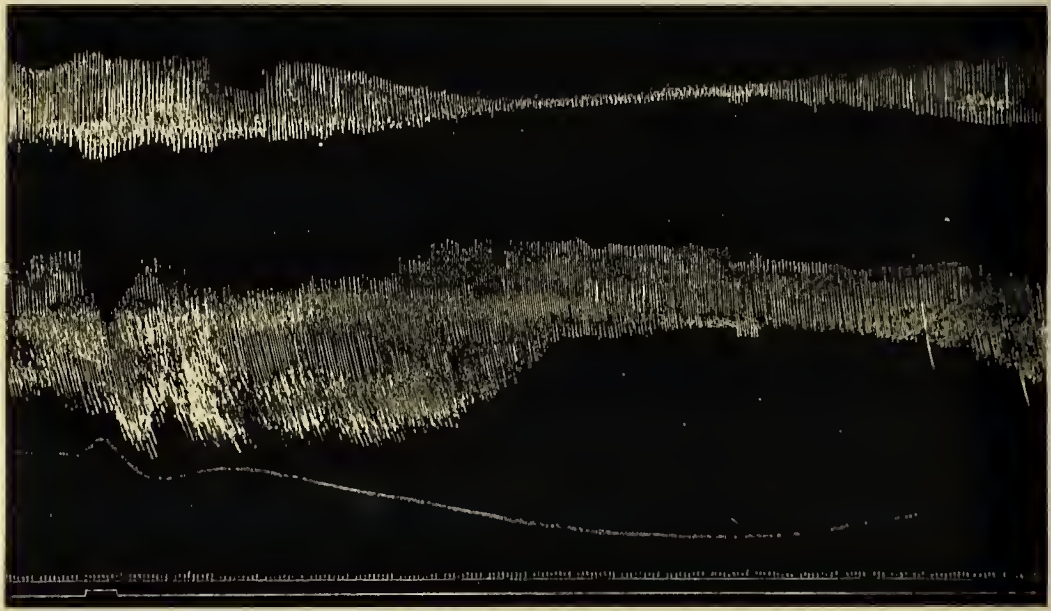


Fig. 5. *Cat.* Artificial respiration. Cardiac levers. During systole the levers move upwards, during diastole downwards. Upper tracing = right auricle, middle = left ventricle, and the lowest = blood-pressure. Time marker = seconds. (Compare with Fig. 19, where the "B" effect has been eliminated.)

Observation during this effect shows that the right heart is considerably over dilated. During this condition on the right side, the left ventricular systole is more complete, but relaxation during diastole is much less than usual. Recovery is very gradual. The effect is mainly auricular and accurately corresponds to the fall in pressure.

EFFECT ON RESPIRATION.

Small injections of orchitic extract in the rabbit produce acceleration in the rate of respiration, with some diminution in the amplitude of the record (Fig. 3). Larger doses give rise to further effects shown in a more accurate way in Fig. 6.

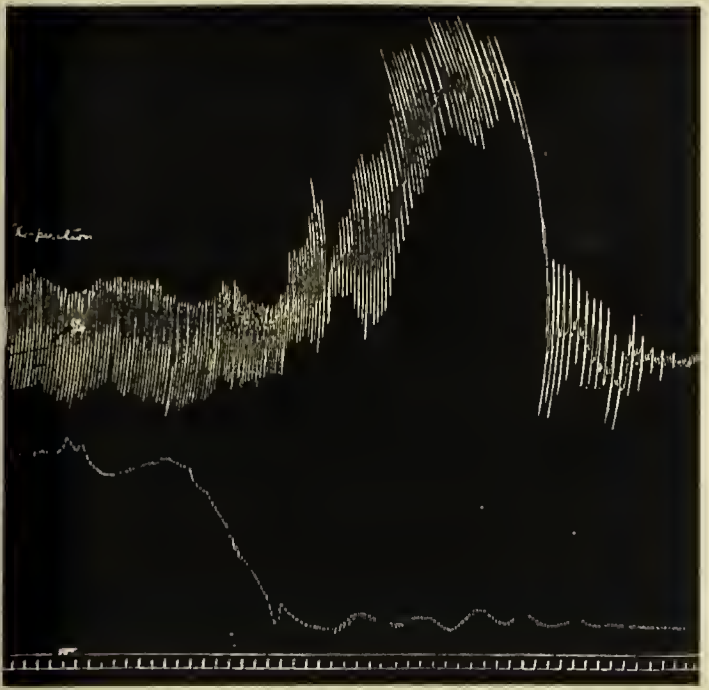


Fig. 6. *Rabbit.* Upper tracing represents the movements of the diaphragm. The diaphragmatic slip fixed to the xiphisternum has been isolated and is connected by a thread over a pulley to a horizontal lever. Shows the effect of injecting an extract composed of half a rat's testis. Time marker = 4 seconds.

Such injections produce at first a quicker but shallower respiration; this becomes gradually slower and gasping in character, the diaphragmatic tonus meanwhile increasing. The diaphragm suddenly relaxes, and after a few spasmodic gasps respiration ceases entirely.

In the cat, after a large but not lethal injection, respiration becomes rapidly shallower and ceases, or there may be no alteration till the cardiac inhibition occurs, when there is an immediate arrest, and then a very slow inspiratory spasm, towards the end of which very shallow respiratory efforts are made; these gradually increase in depth until a condition is reached in which the respiration is both deeper and more rapid than before injection.

These respiratory effects closely resemble the effect produced by stimulation of the central vagus after section of both. Further evidence will be adduced later to show that the effect is a central one produced reflexly through the vagi.

In the dog also, respiration is affected, though much less than in the case of the other two animals: it becomes quicker and deeper than before injection.

ONCOMETER EXPERIMENTS.

Intestine. In the cat intestinal plethysmographic tracings of the effects of orchitic injections show there is a brief initial dilatation of vessels corresponding to the "A" effect; the fall of pressure generally precedes the splanchnic dilatation by a few seconds. If a small injection has been given—that is when the cardiac inhibition is slight and respiration is not arrested—the initial dilatation of vessels is followed about the time of the "B" effect by an increased dilatation of a much more considerable degree than the former. In Fig. 7 such an effect is shown.

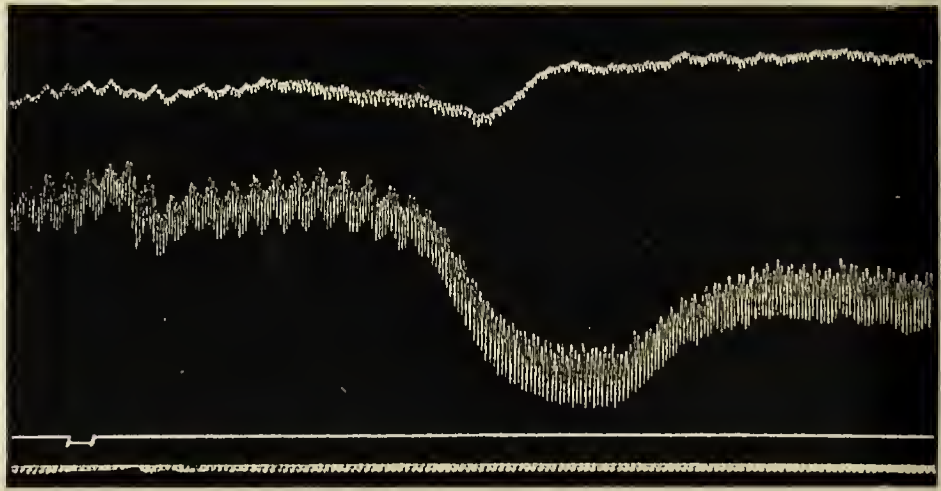


Fig. 7. *Cat.* Volume of a loop of intestine, and blood-pressure. Shows the effect of injecting an extract of half a guinea-pig's testis. Time marker = seconds.

After large injections, that is in those cases where there is marked cardiac slowing, the initial dilatation is quickly followed by a constriction; this effect is only temporary and rapidly changes to a condition of considerable dilatation. As this constriction is only observed after fairly large injections which are generally associated with a considerable fall of blood-pressure, and as it usually corresponds in time to the fall, it might be considered passive, that is a direct result of the cardiac inhibition: but this is not the case, as the constriction rapidly gives place to dilatation whilst the pressure may still remain unaltered. It may be noted here that the amount of injection in Figs. 7 and 8 is the same: this gives an idea of the considerable variability in toxicity of the extract.

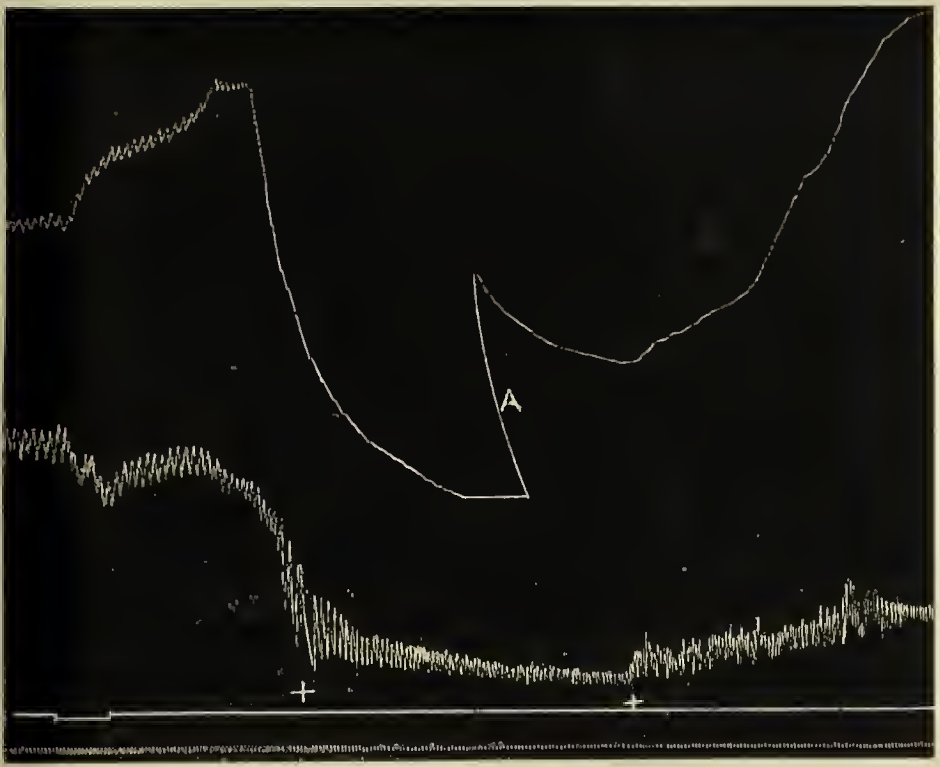


Fig. 8. *Cat.* Intestinal volume and blood-pressure. Shows the effect of injecting a very active extract. The rise "A" is artificial, the recording apparatus having reached the limit. Time marker = seconds. There was complete cessation of respiration during the period marked by the crosses.

Orchitic injections in the dog produce even more marked splanchnic dilatation than in the cat. Dilatation generally begins with the fall of pressure and continues unaltered a considerable time, so that when the blood-pressure has again reached its normal level the splanchnic area still shows evidence of congestion. Constriction of the vessels of the splanchnic area is never observed in the dog.

In the goat the first and smaller rise of pressure is associated with dilatation of the splanchnic vessels and is probably cardiac. The second rise, "B" effect, is obviously vaso-motor. The intestinal vessels gradually constrict and the pressure correspondingly rises until at the point of maximum constriction the blood-pressure reaches its highest level: as the constriction gradually gives place to dilatation the pressure falls. The ultimate dilatation is very marked and sufficient to produce a low blood-pressure (Fig. 4).

In this animal it is evident that the vaso-motor system is much more readily influenced than the heart, and although during the "B" effect cardiac slowing is present yet the constriction of vessels—which is also seen in the cat—is the important factor, and the pressure rises: the rise is but a short one, and splanchnic dilatation, which is the more prominent condition in other animals (dog) rapidly ensues. This rise of pressure in the case of the goat is not peculiar to testis injections only, for—as I hope to show on a future occasion—some drugs which give rise to a cardiac fall of pressure in the cat and dog produce a vaso-motor rise in the goat.

Kidney. The volume of the kidney undergoes a rapid diminution.

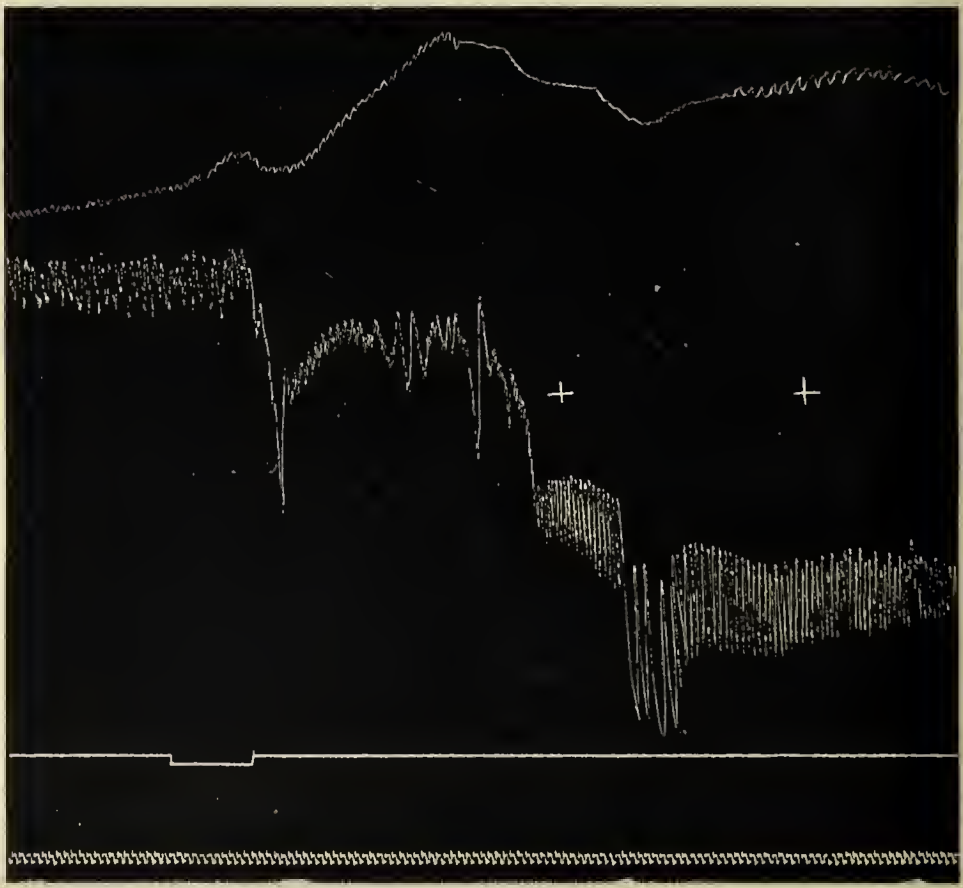


Fig. 9. *Cat.* Splenic volume and blood-pressure. Absence of respiration between the crosses. Time marker = seconds.

This change does not in any way correspond with the blood-pressure and the maximum constriction is reached generally at a point when the pressure is already beginning to rise. In exceptional cases where the blood-pressure undergoes little or no fall, diminution in renal volume is nevertheless evident and the effect therefore must be vaso-motor. After large injections the constriction is much prolonged, frequently lasting ten minutes, but in every case it eventually gives place to dilatation (Fig. 15). It is not easy with this organ to distinguish clearly between the "A" and "B" effects.

Spleen. This organ behaves towards orchitic injections in a very similar manner to the intestines. There is an initial diminution of volume corresponding in time to the "A" fall—an effect which is most probably muscular in view of the simultaneous splanchnic dilatation and the fact that orchitic extract has an exciting effect on mammalian involuntary muscle. This constriction rapidly gives place to a considerable increase of volume which undergoes a temporary diminution about the time of the cardiac inhibition probably as a result of the fall in blood-pressure. Splenic 'waves' are stimulated and frequently continue to be very pronounced for some minutes after injection (Fig. 9).

Limb. An air oncometer was chosen here as in the case of other

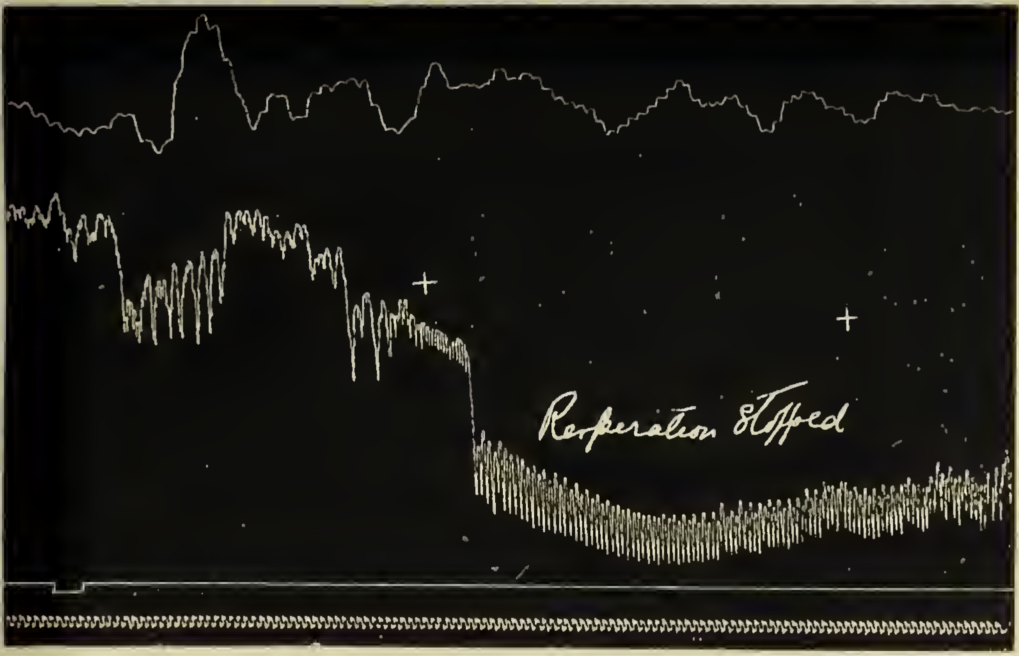


Fig. 10. *Cat.* Plethysmographic record of the hind limb, and blood-pressure. Shows the effect of injecting an extract composed of three-quarters of a rat's testis. Time marker = seconds. There was cessation of respiration between the crosses.

organs. The alterations in the volume of the hind limb of the cat after a moderate injection consist of a primary dilatation corresponding to the initial fall in blood-pressure: the dilatation does not however accurately coincide in time, but like the intestines begins a little late. Occasionally during this latent period a small constriction may occur. This increase in volume though well defined is short and is followed by a series of irregular vaso-motor alterations in volume, the mean volume of the limb remaining however slightly increased (Fig. 10).

Testis. Oncometric records of this organ cannot be obtained satisfactorily from the cat and rabbit. This gland in the dog, as I have shown elsewhere (12), undergoes alterations in volume which are however very small in comparison with those which occur in other organs; and also its vaso-motor system is poorly developed, so that after the exhibition of peripheral vaso-constrictor drugs such as nicotine, the rise in blood-pressure overcomes the tendency of the vessels to constrict, and the testis enlarges.

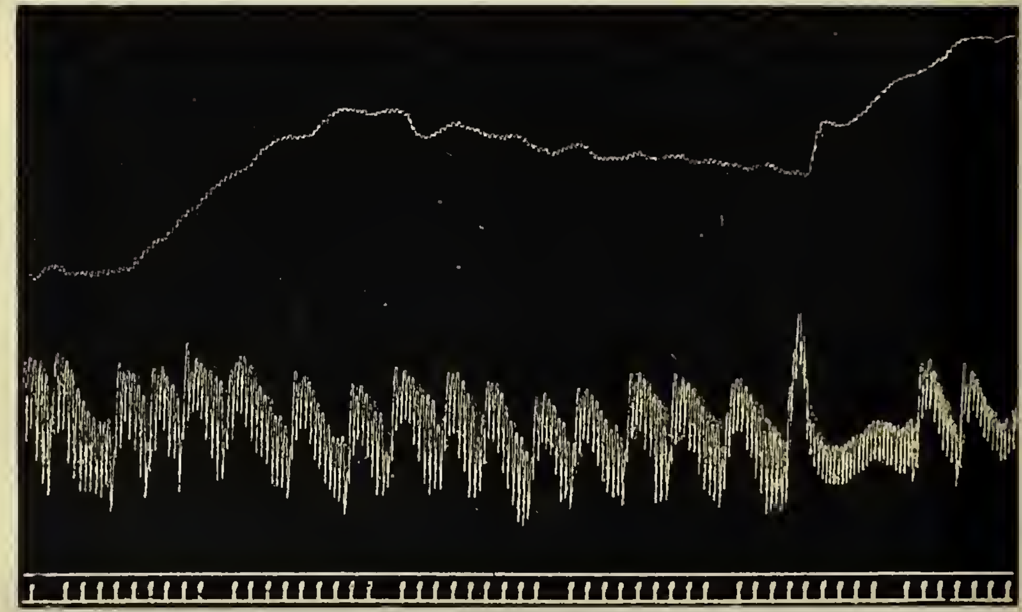


Fig. 11. *Dog.* Testis volume and blood-pressure. A large injection of orchitic extract was given between two and three minutes previously, which produced constriction of the testis. Dilatation began as a series of waves, two of which are shown here. Time marker = seconds.

After injections of orchitic extract the volume of the testis usually shows an initial constriction; this however in all cases is followed by marked dilatation. After large injections the constriction is marked and frequently prolonged, and dilatation when it occurs instead of being gradual may occur as a series of waves, two of which are shown in Fig. 11.

It was considered possible that the initial constriction of the testis might be muscular as in the case of the spleen, and the sudden rhythmical waves of dilatation were taken to support this view. Involuntary muscle has not been found in the dog's testis although careful search has been made [1].

Injections of epididymis extract in the dog produce a very marked and immediate splanchnic and testicular dilatation (Fig. 12). Further reference will be made to this subject later. The effect is somewhat different from that of ordinary orchitic extract and will be considered further.

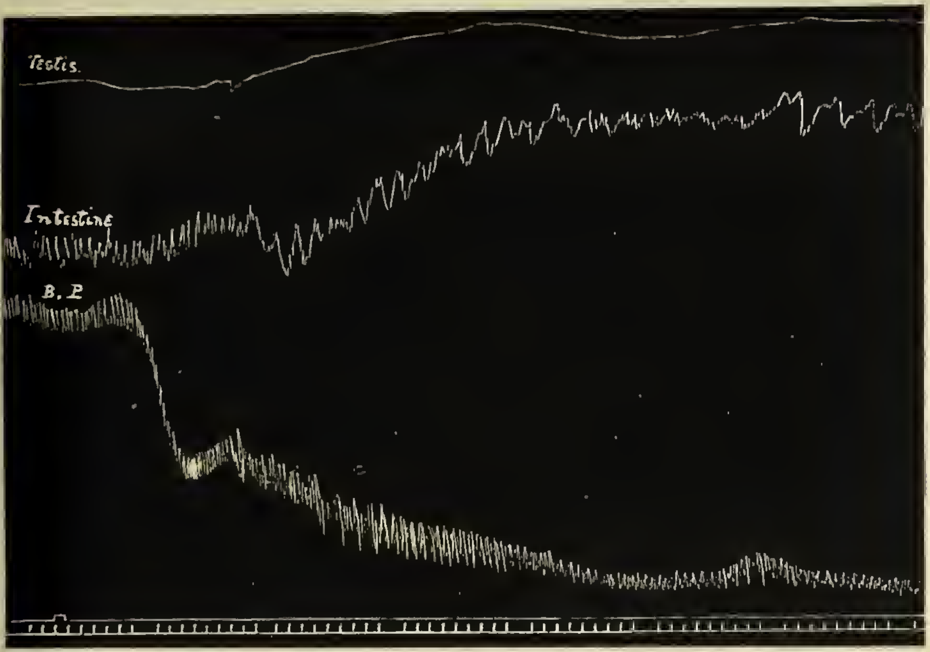


Fig. 12. *Dog.* ·02 grm. morphia. Shows the effect of a large injection of extract of epididymis. Time marker = 2 seconds.

In the goat, where the vaso-motor mechanism of the testis appears to be more highly developed, the effect of orchitic injections is to produce a very well-defined constriction exactly corresponding to the intestinal effect (Fig. 4). The greatest constriction is reached at the maximum rise in pressure. The testis at first dilates slowly, but after about half a minute the dilatation suddenly becomes much more rapid.

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[1] According to Kölliker plain muscle fibres are prolonged upon the septula which are given off from the front and sides of the corpus Highmori.

It will not be out of place here to refer to the direct application of orchitic extract to the mesentery of the frog. During a series of experiments in which the mesentery was carefully exposed and examined with an inch objective, I was unable to observe any decided alterations in the blood vessels after painting with concentrated extract, either tending towards dilatation or constriction. Further evidence dealing with action on vessels will be considered under the heading 'boiled extract.'

JUGULAR AND CAROTID INJECTIONS.

It has been shown already that the latent period between the time of injection and the production of cardiac inhibition is a variable one: sometimes the inhibition occurs as soon as half a minute after injection but it may be delayed to one and a half minutes. The same extract however always gives rise in the same animal to a constant latent period, and it therefore becomes possible to compare this period when the extract is injected into the central end of the jugular vein with that where the injection is made into the peripheral end of the carotid artery. The two injections should be made with the same standard extract and as far as possible under the same conditions, such as rate of injection, etc. A considerable time must be allowed for the recovery of the animal between the injections.

The carotid injection was made in each case into the peripheral end of the same carotid from which the blood-pressure was taken, and the latent period was always longer than after jugular injections, 20 seconds being an average difference between the two; for example, if the jugular injection had a latent period of 50 seconds, the carotid would probably be about 70 seconds.

This latency also applies to the respiratory effect. Some of the injections were made whilst the opposite carotid was temporarily blocked and with one jugular allowed to bleed, these conditions being removed as soon as the injection was actually completed.

These facts appear to suggest a peripheral origin for both the cardiac inhibition and respiratory effect in the cat.

Carotid injections of orchitic extract do not show the fall of pressure "A"; this is an additional reason for believing that the "A" fall is largely cardiac, because when injected into the carotid the active body only reaches the heart much diluted and not in the comparatively concentrated condition as when injected into the central end of the jugular vein.

THE EFFECT OF SECTION OF BOTH VAGUS NERVES.

If both vagus nerves are severed in the cat the injection of an active orchitic extract ($\frac{1}{2}$ to $\frac{3}{4}$ rat's testis) fails to determine the usual result. The "A" effect with all its attendant phenomena is produced in a perfectly typical manner, but there is no subsequent cardiac inhibition and respiration remains unaffected. Blood-pressure frequently shows well-marked Traube-Hering waves (Fig. 13), sometimes it rises slightly, and sometimes falls. Although the pressure is so little altered the effect on vessels is much the same as that which occurs with intact vagi. The renal vessels show decided constriction whilst the splanchnic dilate.

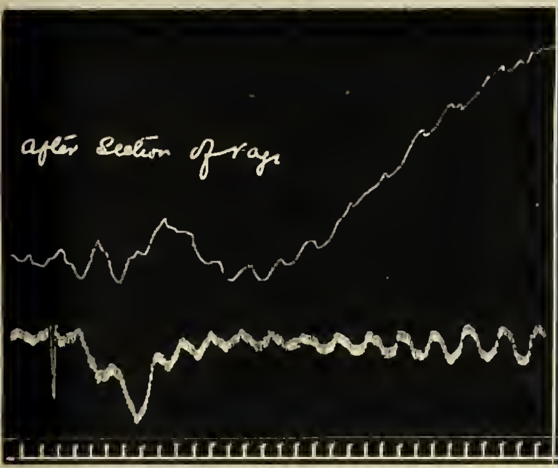


Fig. 13.

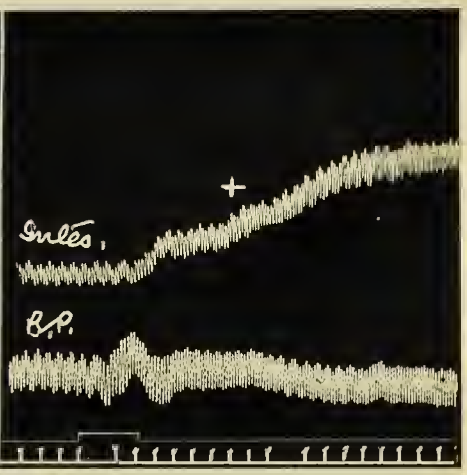


Fig. 14.

Fig. 13. *Cat.* Intestinal volume. Blood-pressure. Shows the effect of a large injection of fresh orchitic extract after section of both vagus nerves. Time marker = 3 seconds. Traube-Hering waves well marked.

Fig. 14. *Cat.* A.C.E. $2\frac{1}{2}$ c.c. $\frac{1}{2}$ °/$_0$ atropine. Effect of a large injection of orchitic extract. Time marker = 2 seconds. (Compare with Fig. 22.)

Fig. 13 shows the blood-pressure and the volume of a loop of intestine after section of the vagi. It will be seen that injection of orchitic extract first increases and then diminishes the intestinal volume, corresponding to the "A" effect, and immediately following this action produces a much more decided and prolonged dilatation.

This evidence would tend to show that the cardiac inhibition is of central origin whilst the respiratory effect is peripheral. Very large injections may still produce cardiac inhibition, respiratory failure and death.

In the dog moderate injections fail to produce the "B" effect as seen in the cat with intact vagi, and as this effect alone is modified by section of the vagi one would not *à priori* expect to obtain any modification. With larger injections, that is where there is a considerable and prolonged fall of blood-pressure, the behaviour is exactly the same before and after cutting the vagus nerves.

ACTION OF NICOTINE AND ATROPINE.

If nicotine be given to the cat until stimulation of the peripheral vagi produces no cardiac inhibition, the action of orchitic injections is modified. The "B" effect is entirely eliminated, that is the cardiac inhibition and stoppage of respiration are absent, but the initial fall of pressure remains unaltered.

Atropine produces a still further effect. The amount of atropine found requisite in the full-grown cat for the production of vagal paralysis without affecting the vaso-motor nerves, the cardiac accelerators, cardiac muscle or the medulla, was from 0·01 to 0·02 grm. Half this amount, 0·005 grm., however was found sufficient to eliminate the cardiac inhibition and respiratory arrest—that is the "B" effect—and although it failed to cut out the initial fall of blood-pressure, it reduced it somewhat.

The injection of orchitic extract into a suitably atropinised cat produces either a small immediate rise in pressure or no effect corresponding to "A." A fall of pressure at this period has not been observed. Subsequently the pressure may continue quite unaltered or may, 40 to 50 seconds after injection, undergo slight alterations in level, generally consisting of a slight rise followed by a gradual fall.

In all cases considerable dilatation of splanchnic area is observed. This begins as a small dilatation which in a time varying from 10 to 30 seconds undergoes a further and more considerable dilatation. The effect is a very prolonged one. Careful consideration of the slight changes in the blood-pressure, previously noted, in comparison with the condition of the splanchnic vessels makes it evident that such slight changes in pressure as occur are due to vaso-motor alterations in this area (Fig. 14).

The injection of larger amounts always induces an immediate fall in blood-pressure which, as will be shown later, is cardiac in origin and can be accounted for by the presence of inorganic salts.

As the condition "B" is dependent upon the integrity of the vagi, and as the result has been shown to be produced peripherally it would seem probable that the condition is one initiated peripherally, and produced reflexly through the centre, both the afferent and efferent impulses, for the heart effect, passing through the vagi.

THE ACTION OF THE BOILED EXTRACT.

For preparing the boiled solution, the normal standard extract was heated just to the boiling-point and then immediately cooled, strained through muslin, and filtered through paper. The action of extracts so prepared corresponded in every way with the "A" effect obtained after the injection of normal extract. There was an immediate fall of pressure with slight slowing, followed by a quick recovery: in connection with this fall there was dilatation of peripheral vessels particularly well observed in the splanchnic area. This dilatation was not usually observed until some seconds after the fall, and it frequently commenced about the period of maximum fall. Plethysmographic tracings of the intestines, limb and testis showed this effect, and the limb vessels subsequently underwent a further irregular series of vaso-motor changes (Fig. 17).

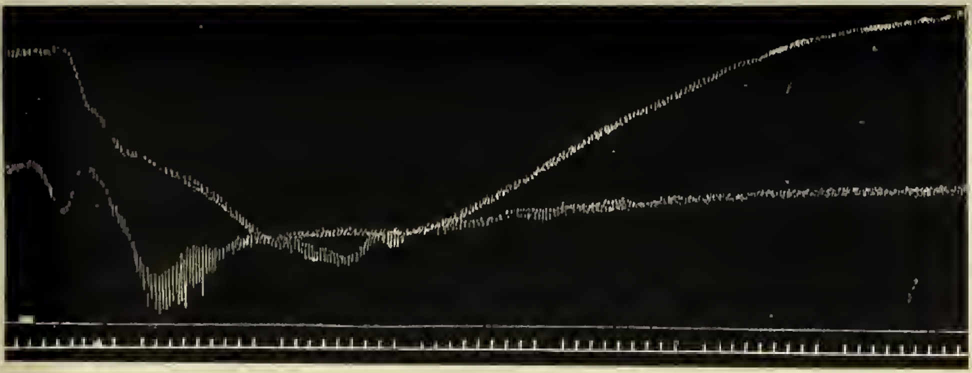


Fig. 15. *Cat.* Kidney volume. Blood-pressure. Effect of an injection of an extract of three-quarters of a rat's testis. Time marker = 3 seconds.

The kidney vessels constricted with the fall of pressure and the maximum diminution in the renal volume generally did not correspond in time to the maximum diminution in pressure; dilatation ensued after the rise, and the ultimate relaxation was well marked (Fig. 16).

A comparison of Figs. 15 and 16 shows clearly that the "B" effect is absent in Fig. 16 and the active substance is therefore destroyed by boiling. A comparison of Figs. 10 and 17 shows the same condition.

Cardiometer experiments showed that during this effect the heart became slightly more dilated with blood than normally and that during the fall in blood-pressure did not contract to so small a size as usual. Recovery of normal tonus was rapid and the blood-pressure simultaneously rose. The later effect showed a condition of greater contractile force with an increased output.

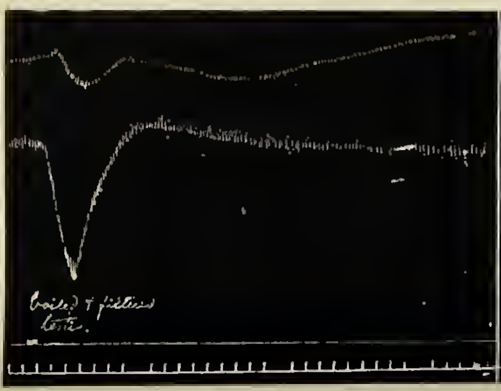


Fig. 16.

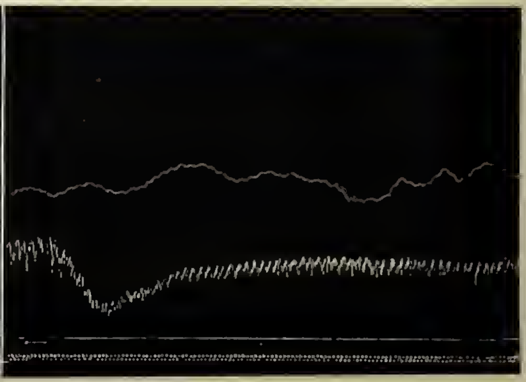


Fig. 17.

Fig. 16. Same animal as in Fig. 15. Fifteen minutes later. Effect of an injection of an extract of one rat's testis (boiled), (compare Fig. 15). Not quite typical, some potassium effect would seem to be present.

Fig. 17. *Cat.* Limb. Blood-pressure. Effect of injection of boiled extract. The extract was the same as that, the effect of which is shown in Fig. 10. These two tracings should be compared. Time record = seconds.

A number of experiments were performed with levers connected directly with hooks fixed in the right auricle and left ventricle of the cat's heart.

Small injections produced a slightly increased relaxation of the right auricle which showed itself only during the period of cardiac slowing, and therefore might be ascribed probably to the increased length of diastole. The force of the auricular systole was also slightly increased, but the whole effect so far described was a trifling one probably dependent on the slowing.

The left ventricle did not behave quite the same, during the slowing, as in the case of the auricle, relaxation of the ventricle was more complete and the systolic force was slightly diminished. Observation showed that during this effect the right heart was slightly over-distended.

Larger injections produced a diminution in force in both the auricle and ventricle.

As an unusual occurrence the effect of injections has been very considerable on the auricle, whilst the ventricle has continued to beat either unaltered or even more vigorously.

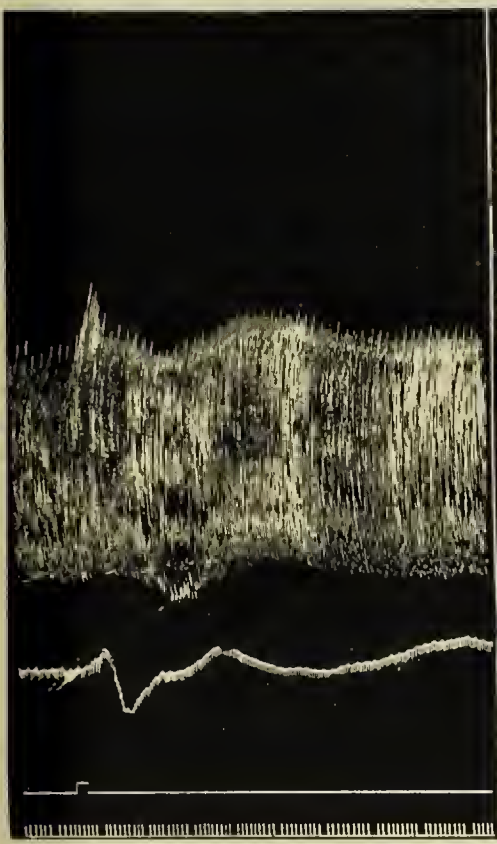


Fig. 18.

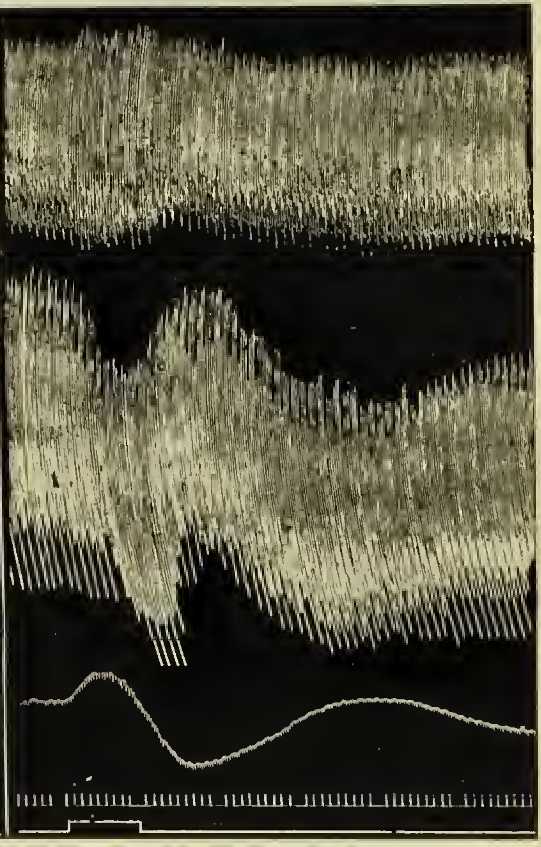


Fig. 19.

Fig. 18. *Cat.* Cardiometer. Down-stroke = systole. Blood-pressure. Shows the effect of a small injection of orchitic extract. (½ guinea-pig's testis.) Time marker = seconds.

Fig. 19. *Cat.* Urethane. Movements of right auricle and left ventricle, recording by Brodie's levers. Rise of the lever represents systole. Blood-pressure. Shows the effect of a small injection of boiled fresh orchitic extract. Time record = seconds. (This figure should be compared with Fig. 5.)

Fig. 20 shows such a case where the squeeze of the ventricle is actually increased—the latter injection was from a dried extract from the ram, the former from the fresh of the guinea-pig.

The action of the boiled extract on the frog's heart corresponded to the action of the extract as a whole, *i.e.* little or no effect could be registered by simply dropping the extract on the heart, but slowing and diminution in the force of the ventricular beat were observed as a result of perfusion. This effect is antagonised to some extent by atropine.

As it seemed probable that orchitic substance administered by the stomach would correspond in action to injections of the boiled extract, some experiments were undertaken to determine this point. These experiments were performed on cats, and urethane was used as an anæsthetic. The cat was given milk containing urethane and was subsequently injected with more until about 1½ grms. per kilo. body-weight had been administered.

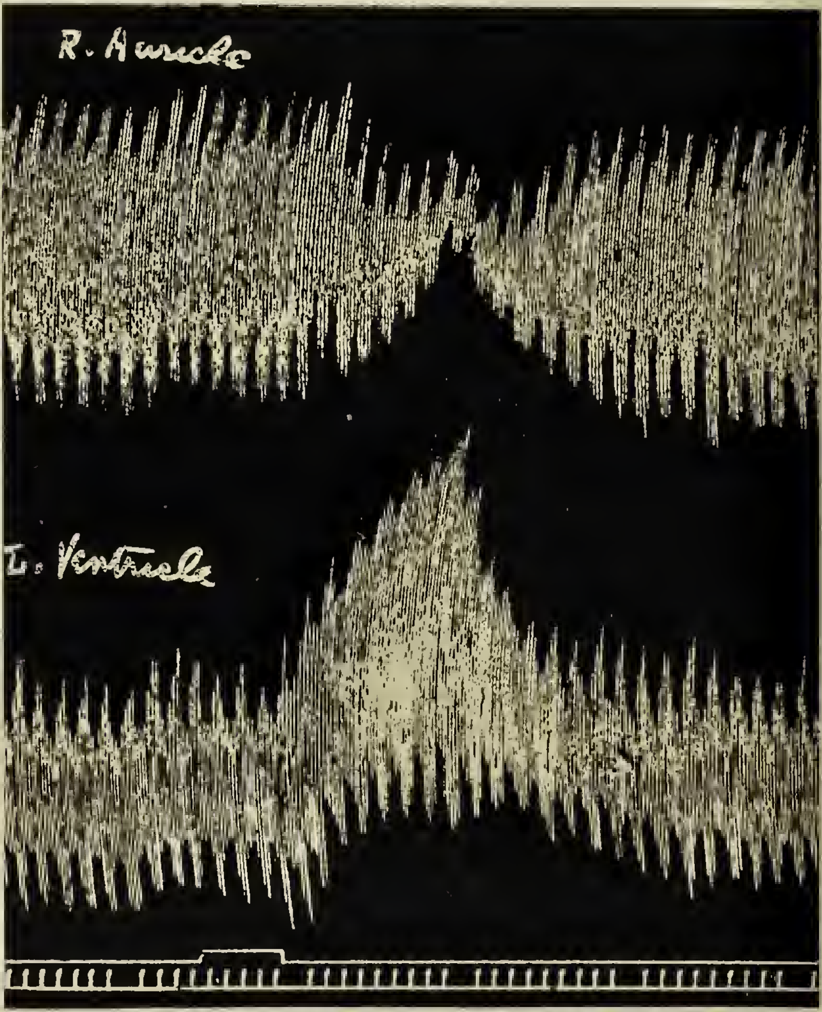
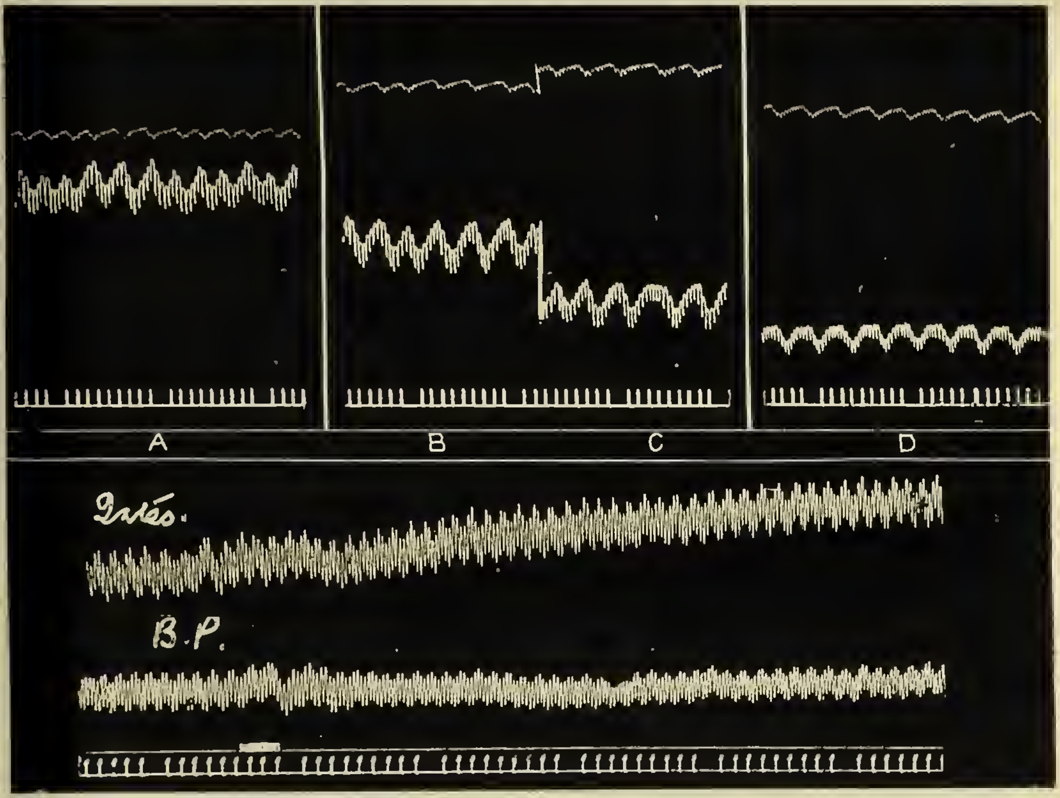


Fig. 20. *Cat.* Urethane. Cardiac movements. Shows effect of a small injection of boiled orchitic extract prepared from the dried ram's testis. Time marker = seconds. (Unusual effect.) Ventricular systole is more perfect, but diastole is less complete.

Arrangements were then made for recording carotid blood-pressure and the volume of a small loop of intestine. The animal was kept in a hot room on a water bath and covered up; short tracings were recorded every ten minutes until both curves had assumed a constant height for twenty minutes. At this point a large amount of orchitic substance pounded up with tap water (40 c.c.) was administered into the stomach by means of a tube. In two or three minutes blood-pressure began to fall and the volume of the intestine to increase, the maximum volume being reached in about twenty minutes. During the dilatation the excursion of the plethysmographic lever due to heart-beats was increased, giving further evidence of the vaso-dilatation. Throughout the experiments no alteration in cardiac rhythm was observed.



Figs. 21 and 22.

Fig. 21. *Cat.* Urethane. Intestinal volume and blood-pressure. "A" shows the state of affairs before the administration of testis. "B" shows the effect 3 minutes after the introduction of orchitic extract into the stomach. "C" the effect 10 minutes after, and "D" 35 minutes after. It is possible that the increased fall of pressure in "D" may be vaso-motor, the apparent constriction of the intestinal vessels being the result of cold. Time record = seconds.

Fig. 22. *Cat.* A.C.E. $2\frac{1}{2}$ c.c. $\frac{1}{2}$ $^0/_0$ atropine. Intestinal volume and blood-pressure. Effect of injection of boiled orchitic extract. (Compare Fig. 14.) Time record = seconds.

Forty c.c. of tap water placed in the stomach of a control animal failed to produce this effect.

Urethane is not an ideal anæsthetic for this purpose as, although it does not affect blood-pressure or peripheral vessels, it inhibits the stomach movements (as will be shown in another communication) and thus retards absorption.

Cutting both vagi or administering nicotine to an animal did not affect its response to boiled orchitic extract.

On atropinised animals the fall of blood-pressure was eliminated, but splanchnic dilatation was still evident.

The splanchnic dilatation produced by the boiled extract on an atropinised animal was less than that induced by injecting a corresponding amount of normal extract.

Fig. 14 and Fig. 22 represent tracings from the same animal, and the same amount of injection was given in each case: in Fig. 14 the normal extract was injected and in Fig. 22 the boiled extract; the splanchnic dilatation is much greater in the former case, and the increased dilatation beginning at the "cross" and evidently corresponding to the "B" effect, is clearly shown.

Much larger injections of boiled extract have a marked effect on the heart, blood-pressure falls almost to zero and oncometric tracings run parallel with the blood-pressure curve. This effect will be further considered under "inorganic salts."

The direct application of orchitic extract to the isolated strip of the frog's stomach arranged so as to pull upon a horizontal lever, sets up a series of small waves.

If the extract or boiled extract is injected into the circulation of a cat peristaltic movements are slightly increased: it has been already shown that orchitic extract "A" substance probably produces some contraction of splenic muscle (Fig. 25). Boiled orchitic extract would therefore seem to have a slight stimulant effect on involuntary muscle (cp. Spermine (15)).

NUCLEO-PROTEID.

Fresh orchitic extract contains a large percentage of nucleo-proteid the action of which was separately determined. An extract of testis was prepared using distilled water, and the nucleo-proteid precipitated with 10 % glacial acetic acid by adding it drop by drop till no more precipitate formed. The precipitate settled in a short time and was then collected, washed with water, removed to a clean vessel and dissolved in the minimum possible quantity of 1 % Na_2CO_3. By this means a slightly opalescent liquid was produced containing the nucleo-proteid alone.

This solution injected into the circulation of a cat usually produced either a gradual slight rise in blood-pressure, or a small fall, but in any case the effect was slight until, at a period varying between 2½ and 5 minutes from the time of injection the pressure gradually fell, the heart became markedly slowed, and respiration ceased. The respiration usually recovered soon, but the inhibition once produced in this way was very permanent and frequently lasted a quarter of an hour before any marked recovery occurred. Artificial respiration was occasionally necessary. An immediate slight fall of blood-pressure with rapid recovery sometimes occurred directly after injection, doubtlessly due to contamination with extractives as the action was not present when the nucleo-proteid was thoroughly washed.

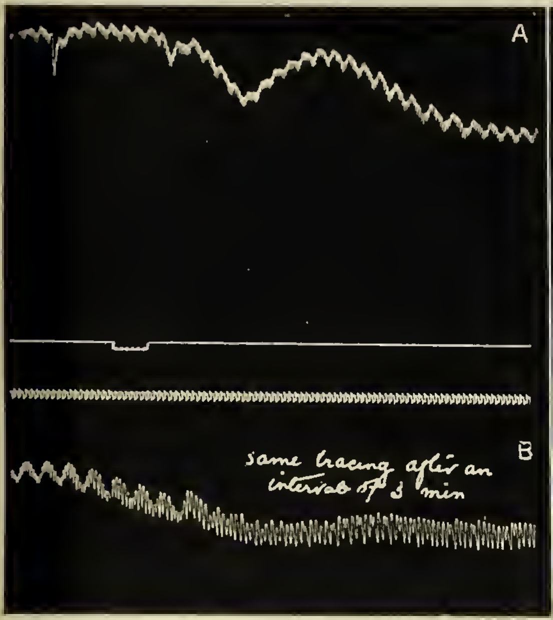


Fig. 23.

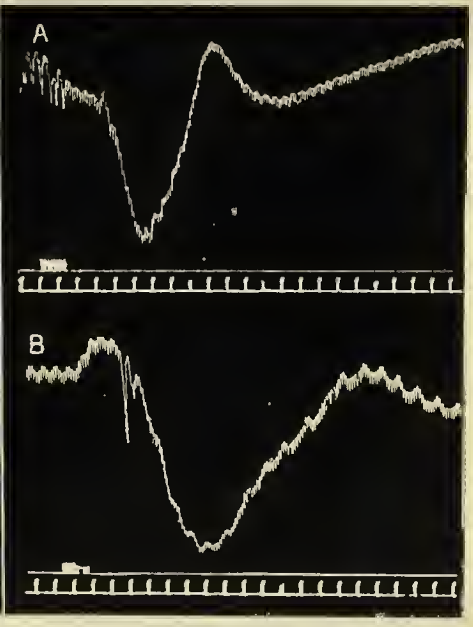


Fig. 24.

Fig. 23. *Cat.* Blood-pressure. Between tracings "A" and "B" there is an interval of 3 minutes. Effect of injection of nucleo-proteid from testis prepared by the acetic acid method. Time marker = seconds.

Fig. 24. *Cat.* Blood-pressure. "A" shows the effect of an injection of 2 c.c. of a $2\,{}^0/_0$ solution of the ash from the ram's testis. "B" represents the same effect after section of both vagi and the administration of atropine. Time record = 3 seconds.

The long latent period before the inhibition is very characteristic in this effect.

Before, and coexisting with the inhibition, the intestines, spleen and testes showed a dilatation of vessels whilst at the same time the kidneys were constricted. These vaso-motor changes were very gradually developed and lasted over a considerable period. After large injections the intestinal vessels however showed a well-marked constriction, very similar to that which occurs as a result of injection of normal extract, and as the blood-pressure tended to rise at this stage the effect must be vaso-motor.

Severing the vagus nerves or administering atropine before injection eliminated the respiratory effect and the cardiac inhibition.

Other effects of the injection of nucleo-proteid were dilatation of the pupils and protrusion of the eyeballs, but intra-vascular clotting never occurred.

Fresh orchitic extract precipitated with acetic acid and immediately redissolved did not produce this prolonged latent period but gave the "normal orchitic extract effect." A frequent repetition of this process however gradually altered the physiological action and prolonged the latent period[1].

The filtrate obtained after precipitating the extract with acetic acid contained only a trace of proteid. When this solution was neutralised with 1 °/₀ sodium carbonate its physiological action was indistinguishable from the action of the boiled extract already described and was unaffected by section of the vagi or the influence of nicotine, but was eliminated by atropine. The amount of sodium acetate present in such filtrates is much too small to produce any appreciable action.

EFFECT ON LEUCOCYTOSIS.

It is well known that various organic substances such as peptones and organic extracts are capable of producing an increase in the leucocytes when taken into the animal body.

Löwit found that such injections into the circulation produced an alteration in number which in the first stage was diminished but subsequently in a later stage was markedly increased. This alteration affected mainly the polynuclear corpuscles.

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[1] Halliburton and Brodie have shown that this repetition of precipitation and solution of nucleo-proteid is accompanied by a chemical, and a physiological change—as shown by the loss of power to produce intravascular coagulation of the blood in the rabbit.

In my experiments I have never observed intravascular coagulation, the explanation probably being that different animals were used, and in those cases where rabbits were employed the injections were very small. (This *Journal*, XVII. 135.)

Goldscheider and Jacob⁽¹³⁾ among other observers showed that the diminution in the white corpuscles in the first stage was the result of the altered distribution, and that in the capillaries of the internal organs, especially of the lungs, the number was increased.

Since then a considerable mass of literature has accumulated dealing with the effect of nucleo-proteid and its derivatives on metabolism. The following experiments were thought worthy of recording on account of the profound effect produced on leucocytes and the fact that in each case the injections were subcutaneous.

Rabbits were in all cases employed.

The following table shows the effect on the number of leucocytes in the blood of the peripheral circulation before and after the administration of a large subcutaneous injection of orchitic extract prepared from the dried testis of the ram.

Rabbit, weight 2250 grms.

Time	No. of leucocytes per cu. mm.	Remarks
10 a.m.	7000	After feeding.
10.30 ,,	7200	A rather large percentage of leucocytes.
10.40 ,,	——	15 c.c. orchitic extract injected subcutaneously.
12.10 p.m.	1250	Marked hypoleucocytosis.
2.40 ,,	1870	
5.20 ,,	2500	
8.0 ,,	3750	Death occurred between 12 p.m. and 8 a.m.

The important effect to notice is the marked hypoleucocytosis which is rapidly attained, and the very prolonged character of the condition. With smaller injections recovery to the stage of hyperleucocytosis is more rapid but in every case the leukopænia is very prolonged. Fresh orchitic extract from the guinea-pig gives identical results; in one case an extract of a single testis reduced the number of leucocytes in the peripheral circulation from 6000 to 1200 per cu. mm. in five hours, the animal recovering.

To compare with this effect experiments were performed with corresponding amounts of boiled extract and also with spermine and one experiment with each is tabulated below.

Rabbit, weight 2850 grms.

Time	No. of leucocytes	Remarks
10.30	6200	
10.45	——	An inj. of 15 c.c. boiled orch. ext. given.
11.45	3500	Hypoleucocytosis.
2.25	6200	
4.7	8750	Slight hyperleucocytosis.
5.25	7200	

Rabbit, weight 2020 grms.

Time	No. of leucocytes	Remarks
10.45	6600	An inj. of 2 c.c. Spermine given, ·05 grm.
11.10	——	
11.45	7100	
12.45	8000	Slight hyperleucocytosis.
2.35	7340	

Larger injections of spermine produce a hypoleucocytosis before the 'hyper' condition.

From these figures it is evident that the nucleo-proteid is responsible for the marked hypoleucocytosis which is produced by injections of orchitic extract, as moderate hyperleucocytosis occurs early, that is, in about two or three hours, with corresponding amounts of boiled extract or with spermine[1].

All the leucocytes are affected in this diminution, especially the various granular varieties. In the stage of hyperleucocytosis the increase mainly consists of the amphophil corpuscles.

The appearance of the leucocytes is profoundly altered as shown in films prepared about four hours after injection. The granular corpuscles are frequently increased in size and present an irregular shape and ill-defined outline, the nuclei appear to be much enlarged and take a paler tint than normally with methylene blue.

The granules are less compact and often considerably diminished in number. These changes correspond very closely to those obtained by Milroy and Malcolm(14) after injections of nucleic acid.

Small pieces of lung and other tissues were fixed in corrosive sublimate and imbedded in paraffin and sections showed a small increase in the blood corpuscles in the pulmonary capillaries[2].

THE INORGANIC CONSTITUENTS OF THE TESTIS.

Before drawing conclusions as to the cause of the physiological effect of large injections of testis, it was considered desirable that the quantity and action of the inorganic salts should be determined.

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[1] It is now well authenticated that feeding on nucleo-proteid produces hyperleucocytosis and an increased excretion of P_2O_5. Chabrier has shown that orchitic injections in man produce a diminished excretion of P_2O_5, a fact of some interest in relation to the hypoleucocytosis.

[2] Further experiments are now in progress on this subject.

For analytical purposes the testes of the ram were used; one pair of fresh testes were found to weigh 560 grms. They were sliced into small pieces and thoroughly dried in a hot air oven at 100° F. during three days, when their weight was diminished to 75 grms. *i.e.* about 87 % of the normal testis is water; with the testes of the guinea-pig and rat the percentage was still greater.

Ten grams of this dried material were powdered up and completely incinerated at as low a temperature as possible: after cooling in a desiccator this weighed 0·756 grm, that is to say the inorganic salt forms 7·56 % of dried material. The ash is readily soluble in water, except for a very small percentage of lime, and chlorides, sulphates, phosphates and potassium can be detected by the usual chemical methods.

For experimental purposes a 2 % solution of this ash dissolved in normal saline was employed. Three c.c. injected into the circulation of a cat produced a rapid fall of pressure with slight cardiac slowing followed by a quick recovery. This effect was still obtained with little alteration in the atropinised animal.

Larger injections produced a fall of pressure nearly to zero, the heart stopped beating, and respiration also ceased soon after.

Further experiments with the salts were made by perfusing through the excised frog's heart. The heart was rapidly affected and stopped in diastole. The action on frog's muscle and mammalian heart also produced effects harmonising with the supposition that potassium was the active substance.

Inorganic salts cannot be responsible therefore for any of the effects described, except in those cases where very large injections of boiled extract (especially where the dried material was used for preparing the extract) were employed, when the effect corresponded to the potassium effect and remained uninfluenced by atropine.

THE CHEMICAL COMPOSITION OF ORCHITIC SUBSTANCE.

Normal fresh orchitic extract is alkaline to litmus. Decomposition is very rapid, and a smell of H_2S is eliminated.

Fresh testis contains about 13 % of solid matter in the ram, but less in the guinea-pig, rat and rabbit. Of the proteid matter, nucleo-proteid forms by far the largest bulk, a trace only of other proteid being present. Fibrinogen, proteoses and peptones are absent. With regard to non-proteid organic substances probably a considerable number are present.

Spermine phosphate is easily prepared from semen or epididymis by precipitating with alcohol, filtering, and extracting the precipitate with distilled water containing a drop of ammonia; on evaporating this to dryness on a watch-glass characteristic crystals are obtained. From the testes of the dog these crystals may be obtained without much difficulty by making an extract with water containing a drop of ammonia, evaporating to dryness and removing the fat with ether. The testes of the ram are very difficult to deal with on account of the fat they contain. Spermine gives the usual alkaloid tests; it may be precipitated with phospho-molybdic acid, phospho-tungstic acid, tannin gold chloride, platinic chloride, etc. The gold salt crystallises in irregular golden yellow plates which are characteristic. These plates are of little use however in detecting minute quantities of spermine. Spermine is insoluble in alcohol and ether.

The only other bodies which I have been able to recognise in the crystalline state are cholesterin and kreatin. Choline was not detected.

An alcoholic extract of the dried and powdered gland was obtained by extracting with absolute alcohol during 24 hours: the fluid was then strained off, filtered, evaporated to dryness and taken up with normal saline. The injection of this extract into animals gives rise to effects indistinguishable from those of the "A" effect observed with the normal extract.

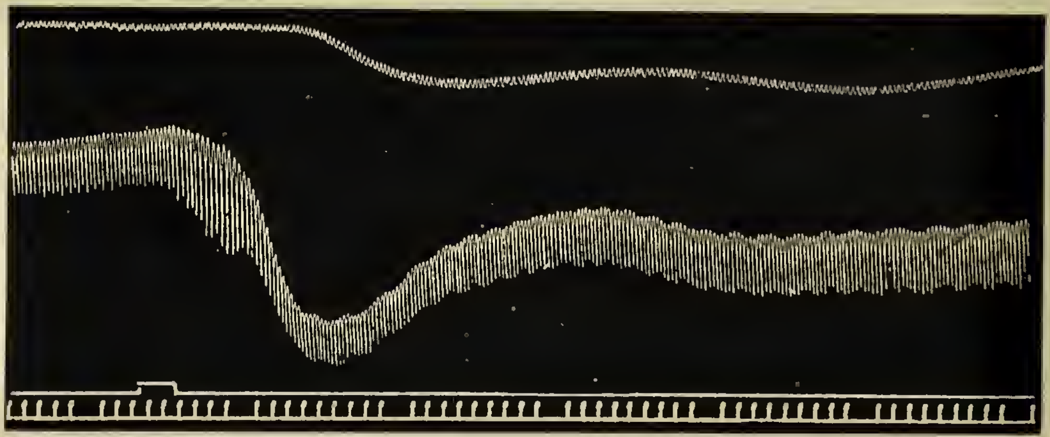

Fig. 25. *Cat.* Urethane. Splenic volume. Blood-pressure. Effect of a small injection of alcoholic extract of testis. Time marker = seconds.

If the material which has been exhausted with alcohol is dried and further extracted with normal saline, a fall of pressure is still obtained of the same character as that given by the alcoholic extract so that at least two depressor substances must be present, one soluble in cold water, and insoluble in alcohol, and the other soluble in alcohol: to the former group spermine belongs.

Further there is a distinct difference in the action of the testis and its secretion (*i.e.* extract from the substance of the epididymis and vesiculæ seminales), in proportion as the latter loses its cardiac effect it gains in its power as a vaso-dilator. This effect is shown in Fig. 12 in the case of the dog and is also seen in the cat. The fall of pressure in both cases is largely vaso-motor. All the organs of the body dilate with the exception of the kidney, which becomes markedly constricted.

Hence it may be concluded that these active bodies inducing vaso-dilatation are produced by the metabolism of the testis (nucleo-proteid). As regards actual recognition, spermine although present in both is much more readily detected in the epididymis.

CONCLUSION.

From the experimental evidence brought forward it is shown that orchitic extract, prepared by macerating the fresh gland with physiological saline, contains three groups of bodies: (1) proteid, (2) organic substances unaltered by boiling, (3) inorganic salts. Of the proteids, nucleo-proteid is the most plentiful and the only one which produces any marked action.

It produces, as observed in solution in normal orchitic extract, a fall in blood-pressure after a somewhat lengthy latent period; this effect is mainly the result of cardiac inhibition and is most highly developed in the cat. Inhibition lasts a time, varying with the amount injected, and recovery is very gradual. Small injections produce a quickening of respiration, but quantities sufficient to obtain well-marked cardiac inhibition cause a temporary absence of all respiratory movements. Respiration begins again gradually and ultimately assumes a condition both quicker and deeper than that which was present before injection. This effect is associated with some dilatation of the splanchnic area, spleen and testis, but the kidney always shows a very considerable and prolonged constriction.

In cats the injection of normal orchitic extract produces, about the time of cardiac inhibition, an initial splanchnic constriction, but this condition, which is not observed in dogs, rapidly gives place to the typical dilatation; in the goat this initial constriction produces a rise in blood-pressure because the associated cardiac effect is absent in this animal.

Both the cardiac and respiratory effects are absent if the vagi have been previously severed, and the effect would therefore seem to be central in origin. If injections are made directly up into the brain through the peripheral end of the carotid artery, the latent period, from the moment of injection up to the time of cardiac inhibition, is prolonged. These effects (cardiac inhibition and cessation of respiration) are therefore probably reflex originating in the periphery, the impulses in the case of the heart passing to the brain and down the vagi.

Splanchnic dilatation still occurs after section of the vagi or injection of atropine.

It will not be out of place here to refer to the opposition of the renal vessels and occasionally the testicular as opposed to the intestinal. For example, in the dog orchitic injections produce intestinal dilatation of vessels accompanied by renal and testicular constriction.

It is difficult to conceive how such an effect can be peripheral, and in a number of perfusion experiments which I have performed with orchitic extract, I obtained identical results whether perfusing the dog's kidney or rabbits' hind limbs[1].

The suggestion of Brodie(16) that this condition is brought about by an excitation of the vaso-motor centre seems the most probable.

The precipitated and redissolved nucleo-proteid has a slightly different action from the native substance in orchitic extract. The latent period is prolonged and the cardiac inhibition when once obtained continues for a very long period.

Injections of nucleo-proteid produce protrusion of the eyeballs and slight dilatation of the pupils but death never results from intravascular coagulation of the blood.

Injections of testicular substance give rise to a prolonged hypoleucocytosis followed by hyperleucocytosis. The hypoleucocytosis is largely the result of altered distribution of the leucocytes, and mainly affects the polynuclear corpuscles. This effect is principally due to the nucleo-proteid.

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[1] There can be little doubt that the testis constriction is muscular, as this organ is in the dog very passive, even dilating after suprarenal injections.

The effect of the second group of bodies may be observed after either section of the vagi, the administration of the filtrate from the boiled extract, or the administration of the gland by the stomach. By any of these methods the nucleo-proteid effect on the heart and respiration is eliminated. The action of this group of substances corresponds closely to the action of a number of leucomaines of which choline may be taken as an example; some small differences have however been pointed out. The effect "A" differs from choline in that whilst with the former substances splanchnic dilatation is not immediate and is not manifest frequently until the maximum fall of pressure is reached, with choline the dilatation is synchronous with the fall. In the former the effect is mainly cardiac, in the latter mainly vaso-motor.

Spermine is responsible partially but probably only in a small degree for this effect because an alcoholic extract of testis—in which spermine is insoluble—produces the same effect. Orchitic injections produce similar effects on animals which have been castrated some months previously and on the normal animal.

Inorganic salts are present to 7·5 % of the dried ram's testis and chlorides, sulphates, and phosphates of sodium, potassium, or calcium can be detected in the ash.

The vaso-dilator substances which are not destroyed by boiling are more powerful in the epididymis and vesiculæ seminales than in the testis and hence are probably derived from the metabolism of the glandular epithelium of the testis.

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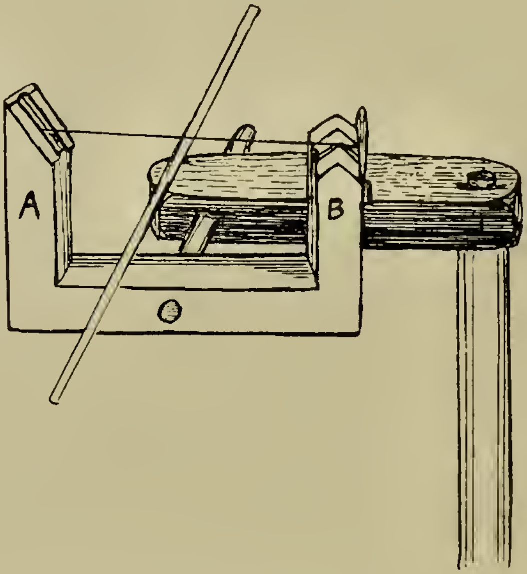
[*From the Proceedings of the Physiological Society*, January 18, 1902.]

A simple form of frog-heart-lever suitable for class-work. By T. G. Brodie.

It must be the common experience of most teachers that the tracings of the frog's heart-beat, recorded by the suspension method in class, are usually most unsatisfactory unless one is able to select large male frogs. This is chiefly due to the recording lever, which usually possesses a considerable amount of friction and is too heavy. To meet these difficulties I have devised a special lever, which has proved so satisfactory that I give the following brief description.



The lever is a long light straw and its axis is an ordinary needle transfixing it. A loop of fine silk is tied to the end of the short arm of the lever and a writing-point is fixed to the other end. The needle forms the axis and simply lies in grooves upon a brass piece shaped as in the figure. The one vertical, *A*, is bevelled off and has a V-shaped groove cut in it. The other vertical, *B*, is bevelled off on both sides and then V-grooves are cut across the centres of the two bevelled faces. To the outer side of *B* a thin steel plate is screwed. The heart having been exposed an entomological pin bent into an S-shape is hooked into the apex. The loop on the lever is passed into the other bend of the pin, and the needle then dropped into its supports. When properly in position the eye end of the needle rests against the steel plate, the needle, just past the eye, lies on the doubly-grooved end, and the point of the needle in the shelving groove of the opposite support. This arrangement has very little friction and is easily managed. The groove in the bottom of which the point of the needle engages shelving, enables one to use needles of slightly varying lengths and, to allow the needle to be brought horizontal, the needle support is fitted to a rod held by friction in a firm support in which it may be rotated. I find that the lever answers very well in class work, and that quite satisfactory tracings are obtained even with small and weak frogs. The writing-point is best made of very flexible paper.

The lever has the further advantages that it cannot well be damaged and is inexpensive.

[*From the Proceedings of the Physiological Society*, July 5, 1902.]

A method of equalising the "strength" of make- and break-induction-shocks. By F. S. LOCKE.

So early as 1841 Joseph Henry found that if the resistance in the primary circuit of an induction-apparatus was raised (this he effected without diminution of current-strength by placing in it a sufficient number of cells in series) the make-shock became stronger in its stimulating power than the break-shock. That the make-effect became under these circumstances more than equal to the break-effect was due to the falling off through polarisation of the strength of the primary current during closure, an effect to be feared even when so-called constant elements are used[1]. The inconstant factor thus introduced together with the inconveniently large number of cells necessary when fairly strong shocks are required, is probably the reason why it has never been attempted to systematically utilise this method of equalising make- and break-effects. It becomes however free from these defects if the high voltage continuous street-current reduced by a lamp-resistance be used to supply the small primary current required for physiological purposes, and is then found to give remarkably good results, even in the case of tetanisation effected with the ordinary Neef's hammer. This to the best of my knowledge has not been pointed out in spite of the frequency with which induction-coils are now thus worked. The method of testing the equality of make- and break-effects used was the highly delicate one based on the difference in excitability of a frog's leg-preparation to currents in opposite directions when the stimulating electrodes (3 to 4 mm. apart) are placed on the nerve close to a fresh "cross-section." This was usually made by ligature. In this case descending shocks excite much more strongly than ascending ones[1], the result being that under tetanisation by means of an ordinary sledge-inductorium, with iron core and Neef's hammer, supplied say with one Leclanché cell, the threshold of excitation when the as yet only effective stronger break-shocks are descending may lie at more than 40 cm. on the scale, while the threshold with ascending break-shocks may lie at less than 30 cm., the difference being about 15 cm. When the same inductorium is driven by an E.M.F. of 100 volts through an 8 candle-power glow-lamp the difference on the scale between the two positions of the thresholds (these lying between 30 and 40 cm.) in the two cases is found to be only 0—0·6 cm.[2].

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[1] See E. du Bois-Reymond. *Monatsber. der Berliner Akad.* 1862. p. 374 (*Ges. Abh.* I. p. 230).

The difference is usually in favour of the break-shock, but this is not always the case, the opposite being sometimes met with, more particularly when a stronger current is used by substitution of a 16 c.p. for the 8 c.p. lamp. It would seem, as might be expected, that the manner of working of the contact-breaker is the variable factor at work, equality of make- and break-effects, or preponderance of the make-, being often brought about, without anything else being altered, by screwing the adjustable contact further home.

The Helmholtz modification is by no means so effective. With the inductorium above referred to equipped with it, but with the iron core not removed, the thresholds in the two cases were about 5 cm. apart, both lying between 10 and 20 cm. Bernstein's modification (a shunt of low resistance to the primary coil) gave a slightly better result, a single Leclanché cell alone being used, and the iron core not removed. If the street-current and 8 candle-power lamp were used with it a very much better result, as would be expected, was obtained, the thresholds in the two cases being only 0·3—0·6 cm. apart, lying between 15 and 20 cm.

The great advantages of the above-described mode of application of

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[1] See P. Grützner and P. Moschner. *Arch. f. d. ges. Physiol.* XXVIII, p. 130, 1882, and E. Hering, *Sitzb. d. Wiener Akad.* LXXXV (3), p. 237, 1882.

[2] I have not had at my disposal an inductorium graduated in relative integral current-strengths. In the one used by me the length of the primary and secondary coils was 9·2 cm. The zero of the scale corresponded to the secondary being pushed quite over the primary coil. Only preparations from uncooled summer-frogs have hitherto been made use of. The electrodes were of platinum.

Henry's method are its ease when the continuous street-current is available, and that, as is often required particularly for the stimulation of muscle, the strength of shock obtainable is not diminished. It does not of course annul the danger of unipolar current-escape as do Helmholtz's and Bernstein's modifications, for instead of the break-shock, as with these, becoming assimilated in character to the make-shock, the reverse is the case. The break-shock itself however is not unaffected, but is prolonged in duration, as is shown by the fact that a series of break-shocks of increasing strength can be made to give a 'Gap' by the introduction of sufficient resistance into the primary circuit[1].

[1] See F. S. Locke. *Proc. Physiol. Soc.* p. xxxix. 1901. (This *Journal*, vol. xxvi.)

THE HEART AND NERVOUS SYSTEM

THE
Heart and Nervous System

BEING

THE HARVEIAN ORATION

Delivered before the Royal College of Physicians of London
on October 18, 1902

BY

DAVID FERRIER, M.D., LL.D., F.R.S.

Fellow of the Royal College of Physicians; Professor of Neuro-Pathology,
King's College, London; Physician to King's College Hospital,
and the National Hospital for the Paralysed and Epileptic

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1902

TO

SIR WILLIAM SELBY CHURCH, BART., M.D.
PRESIDENT OF THE ROYAL COLLEGE OF PHYSICIANS,
LONDON.

THIS ORATION

DELIVERED AT HIS REQUEST

IS DEDICATED

AS A MARK OF ESTEEM.

The Harveian Oration,

1902.

Mr. President and Gentlemen,—I am certain I am but truthfully expressing the feelings of this important assemblage of the College of Physicians when I say that we one and all rejoice that our most exalted Fellow, His Gracious Majesty King Edward, has been happily restored to health and anointed King over a loyal and united nation—united as it has never been before in the history of our race. Our sovereign owes much to the medical profession, for twice, at least, he has been rescued from imminent

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Note.—In the preparation of this address I have freely availed myself of the rich mine of material contained in the works of Tigerstedt *Die Physiologie des Kreislaufes*, 1893), von Bezold (*Die Innervation des Herzens*, 1863) and the well-known text-books of Foster and Schäfer. In particular I gratefully acknowledge much valuable help and counsel from my colleague, Dr. T. G. Brodie.

peril by the skill and devotion of our brethren. His Majesty has been singularly fortunate in having had on both occasions, as officers of his medical body-guard, the greatest living experts in the class of diseases by which he was assailed; and it is to us a legitimate source of satisfaction that our art has made such progress within the memories of us all as to have rendered it possible, in circumstances formerly almost hopeless, to save a life so precious to his subjects. If the King owes much to the medical profession, we on our part, gladly acknowledge the deep debt of gratitude which he has earned at our hands. He has at all times exhibited a sympathetic interest in our work, our needs and aspirations, and in particular by the magnificent endowment which he has secured to the metropolitan hospitals, he has done much to promote the cause of medical education and the welfare of his people. The illustrious Harvey, whose name, on this day of St. Luke, we are met to honour, was also fortunate in having in King Charles a sovereign deeply interested in scientific progress, who provided his trusted physician with many facilities for his observations and experiments, and often in person attended his demonstrations. It was to the king that Harvey dedicated his immortal treatise " De Motu Cordis " in poetical terms of comparison between the heart and body and the soverign and body politic.

> " The heart of animals is the foundation of their life, the sovereign of everything within them, the sun of their microcosm, that upon which all growth depends, from which all power proceeds."

It would be as difficult to understand vital functions, the conditions of health and disease, apart from the circulation of the blood, as it would be to explain the movements of the heavenly bodies without the law of gravitation. Harvey, as has been truly said, is the Newton of physiology. His " Exercitatio " is such a complete and closely reasoned proof of the circulation, that in the 274 years that have elapsed since its publication nothing of material consequence has been added to the cogency of his arguments ; and his demonstration of the movement of the blood in a circle is a model of exposition which cannot be surpassed by any lecturer on physiology at the present day. Harvey's work deals essentially with the mechanics of the circulation, and in this he practically reached finality. But he naturally pondered over many cognate problems which have continued to exercise physiologists and psychologists to the latest times. It was evidently his intention to deal with these in a separate treatise, for, among other things, in his second letter to Riolan, he says :—

> "We also observe the signal influence of the affections of the mind when a timid person is bled and happens to faint. Immediately the flow of blood is arrested, a deadly pallor overspreads the surface, the limbs stiffen, the ears sing, the eyes are dazzled or blinded. . . . And what indeed is more deserving of attention than the fact that in almost every affection, appetite, hope or fear, our body suffers, the countenance changes, and the blood appears to course hither and thither. . . . But here I come upon a field where I might roam freely and give myself up to speculation. And indeed such a flood of light and truth breaks in upon me, occasion offers of explaining so many problems, of resolving so many doubts. . . . that the subject seems almost to demand a separate treatise. And it will be my business in my "Medical Observations" to lay before my

reader matter upon all these topics which shall be worthy of the gravest consideration."[1]

Harvey's speculations on these and kindred subjects, if his "Medical Observations" had come down to us, would have been of exceptional interest, though we cannot doubt that in the light of modern research they would have appeared but inchoate, as our own will doubtless so seem to those who come as many years after us as we are from Harvey. For the language of the physiologist is but a reflex of the state of knowledge of his day, and his explanations do not transcend those of the sciences on which his own is based. In his exposition of vital phenomena, Harvey shows himself still trammelled, and unable altogether to emancipate himself from the notions of Aristotle and Galen. He speaks, like Galen, of the heart as being an "elaboratory, fountain and perennial focus of heat" (second epistle to Riolan):—not, however, in virtue of its proper substance, but because of its contained blood. Though he scouts the idea of the calidum innatum as transcending the qualities inherent in the living heart and blood, or attributable to anything "of a sublime, lucid, ethereal, celestial, or divine nature," he naturally, in the then state of scientific knowledge, failed to master the problem of the source of animal heat. This was only possible more than 200 years after his time by the discoveries of Black, Priestley, Lavoisier, and others on the nature of combustion, and by the researches of modern physiologists on the metabolism of the tissues, and the foci of heat production in the body. We now know that there is no more innate heat in the heart than is evolved by its own intrinsic muscular contractions. Neurology in Harvey's time consisted largely in speculations as to the seat of the rational soul, and the mode of generation and distribution of the animal spirits to the various organs of the body. Little or nothing was known of the relations of the heart to the nervous system. Harvey had, however, some obscure conceptions as to the difference between "natural" and animal motion. He says :—

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[1] Harvey's Works by Willis, p. 129 — slightly transposed.

> "There are some actions and motions the government or direction of which is not dependent on the brain, and which are therefore

> called 'natural.' . . . Many animals are endowed with both sense and motion without having a common sensorium or brain, such as earth-worms, caterpillars of various kinds, chrysalides, &c. So also do certain natural actions take place in the embryo, and even in ourselves, without the agency of the brain."[1]

But he looked upon all these "natural," visceral, or reflex, actions as proceeding from the power of the heart and dependent on it, while the animal motions alone necessitated the controlling influence of the brain. During Harvey's lifetime, however, Thomas Willis was laying the foundations of a more exact neuro-physiology. His work on the descriptive anatomy of the brain, the arrangements of its blood-vessels, the distribution of the cranial nerves, and the relations of the vagus and intercostal, or sympathetic nerves to the thoracic and abdominal viscera contains much of enduring value. Amid much fanciful speculation as to the functions of the different parts of the brain and the nature of the animal spirits, he came near formulating a true conception of the nature of reflex action ; and in particular he clearly differentiated between the nerve centres governing the viscera from those which regulate the functions of animal life. He regarded the cerebellum as the centre of the visceral functions, a view which he was led to adopt, mainly by the anatomical connections which he thought he could trace between this organ and the vagal and sympathetic nerves.

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[1] On Generation—Harvey's Works by Willis, p. 432.

> "When some time past I diligently and seriously meditated on the office of the Cerebel, and revolved in my mind several things concerning it, at length, from Analogy and frequent Ratiocination this (as I think) true and genuine use of it occurred : to wit, that the Cerebel is a peculiar fountain of animal Spirits designed for some work and wholly distinct from the Brain. The office of the Cerebel seems to be for the animal Spirits to supply some nerves by which involuntary actions (such as the beating of the heart, easie Respiration, the Concoction of the Aliment, the protrusion of the Chyle and many others), which are made after a constant manner unknown to us, or no, are performed."[1]

Willis, as we know, was wrong as to his localisation, but in this and other passages he not obscurely foreshadowed the distinction between the splanchnic and somatic systems of nerves rendered more precise in recent years.

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[1] "The Anatomy of the Brain," chapter xv.

Harvey knew that the heart of a cold-blooded animal would continue to beat when removed from the body, and even when cut to pieces, each portion continuing to execute its own rhythmical contractions. Though he does not specially discuss the cause of the heart-beat, yet one may gather from many passages in his writings, that he considered the heart to be endowed with an inherent activity of its own, independently of the brain and nervous system. The discovery, in more modern times, of the ganglia in the substance of the heart by Remak, Bidder and Ludwig, naturally led to the belief that its contractions are conditioned by rhythmical stimuli, proceeding from these intracardiac centres, just as the somatic muscles are normally excited to action by stimuli conveyed from the brain and spinal cord. Though, perhaps, the last word has not been said on this much-debated question, the balance of evidence, largely furnished by the ingenious and skilfully devised experiments of Gaskell, is in favour of the view that the beat of the heart is due to an intrinsic rhythmical contractile power of the cardiac muscle, possessed by all parts in greater or lesser degree, but most marked in the venous sinus and auricle, whence a wave of contraction is propagated through the heart, leading the sequence and setting the pace to the other chambers. From this point of view the cardiac ganglia are merely peripheral ganglia of the efferent visceral fibres of the vagus, analogous to those of the visceral system of nerves in general. The heart, however, in its normal relations is not a mere mechanical pump, automatically working so many times a minute and turning out so many gallons of blood per diem in steady and regular flow. The needs of the organism are ever changing, and the heart and vascular system are ever adapting themselves in accordance therewith. There is scarcely an act, or thought, or feeling which does not reflect itself in the rate of the heart and state of the pulse. The explanation of these variations is to be sought for in the relations which subsist between the central nervous system and the heart and blood-vessels. Though to the physiologist this is a well-worn theme, I trust that in my endeavour to bring into line the various conditions, physical and mental, which influence the circulation, it will not be considered unworthy of my learned audience if I sketch the principal facts and stages in the development of our knowledge of this subject.

The heart and blood-vessels, together with the walls of the hollow viscera, glands and non-striated muscles of the hair bulbs, as well as certain striated muscles derived from the lateral plates of the mesoblast, are innervated by the great system of visceral or splanchnic nerves, the differentiation of which from the somatic, or those which supply the organs and muscles of animal life, is the distinguished merit of Gaskell. And it is gratifying to think that also to other countrymen than Harvey, notably Langley, we owe in large measure what is known respecting the distribution of the ultimate ramifications of this great system of nerves. The visceral nerves consist of two sets, possessed of opposite functions, viz., motor and inhibitory—terms which are applicable to their action on the heart, blood-vessels, and hollow viscera.

The most epoch-making discovery in reference to the innervation of the heart was the observation of the brothers Ernst and Edward Weber[1] in 1845, that electrical irritation of the vagus caused the heart to beat more slowly or to stop altogether for a time in the state of diastole. This fact was an altogether new one in neurology and has been far-reaching in its consequences and application. Through the vagi the cardiac centres exercise a more or less constant restraining influence, so that when they are cut the heart beats more quickly. This effect was observed both by Willis and Richard Lower, but both regarded it as essentially of a paretic nature due to cutting off the main stream of animal spirits. Willis says :—

> "And here it may be rightly inquired into whether the pulse of the heart so necessarily depends on the influence of the animal spirits through the nerves, that it being hindered, the action of the heart should wholly cease? For the decision of this we once made a trial of the following experiment on a living dog. The skin about the throat being cut longways, and the trunk of both the wandering pair being separ-

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[1] *Handwörterbuch d. Physiologie*, Bd. iii., 1846.

> ated apart, we made a very strict ligature, which being done the dog was presently silent and seemed stunned, with a great trembling of the heart."[1]

Willis imagined that the sympathetic fibres of the cardiac plexus were able by a kind of anastomosis to supply the animal spirits after the vagi were divided. The totally different function of the sympathetic was first demonstrated in 1866 by von Bezold,[2] who found that stimulation of the filaments proceeding from the ganglion stellatum—derived, as it has been proved by subsequent researches, from the upper thoracic anterior roots—caused effects entirely antagonistic to those of the vagus, viz., acceleration of the rhythm and increased force of the cardiac contractions. The visceral system also contains sensory or afferent fibres, which are the medium of communication between the viscera and the sensorium, but the mode of origin, course and termination of these nerves are less accurately known than those of the efferent class. The heart, like the viscera, is in normal conditions but poorly endowed

[1] "Anatomy of the Brain," chapter xxiv.

[2] "Die Innervation des Herzens," 1863.

with sensibility, and is barely, if at all, sensitive to tactile stimuli. This fact was first demonstrated by Harvey in the case of the son of Viscount Montgomery, whose heart had been exposed by destructive ulceration of the chest wall in the præcordial region. This interesting patient was brought under Harvey's notice by King Charles. Harvey relates :—

> " I carried the young man himself to the king, that His Majesty might, with his own eyes behold this wonderful case: that in a man alive and well, he might, without detriment to the individual, observe the movement of the heart, and with his proper hand even touch the ventricals as they contracted ; and his Most Excellent Majesty, as well as myself, acknowledged that the heart was without sense of touch, for the youth never knew when we touched his heart except by the sight or the sensation he had through the external integument."[1]

The heart, however, is sensitive to severer forms of stimuli, and more particularly to states of abnormal tension. It is as Budge found[2] more sensitive towards the base, and less so

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[1] " On Generation," Willis's translation, p. 384.

[2] Wagner's *Handwörterbuch*, Band iii., p. 485.

towards the apex. It is generally stated in text-books of physiology that the vagus is the sensory nerve of the heart. This, however, though apparently the case in frogs,[1] in which the vagus and sympathetic nerves run throughout in the same sheath, is not the whole truth as regards man or even animals in general. For after section of both vagi in a cat, Goltz found that pinching, or otherwise irritating, the heart caused reflex movements of the whole body. Though, as has been already remarked, the afferent fibres of the visceral system have not been experimentally determined with the same precision as the efferent, there are many reasons for believing that they are connected with the same spinal segments. This is borne out by Head's brilliant work on the relations between visceral disease and cutaneous pain.[2] Pursuing Ross's hypothesis that the viscera receive their sensory fibres from the same segments of the spinal cord as those from which the somatic sensory roots

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[1] Goltz, *Virchow's Archiv.*, vol. xxviii., 1863.

[2] "On Disturbances of Sensation with Special Reference to the Pain of Visceral Disease." *Brain*, vol. xvi., 1893.

arise along which the pain is referred, he has succeeded in mapping out with marvellous definition the segmental relations of the sensory nerves of the various viscera. From these it appears that painful conditions of the heart refer not only into the regions connected with the vagi, but also into those of the upper dorsal segments whence the heart also receives its chief motor supply. The sensory nerves of the heart, therefore, lie not only in the vagus, but also in the rami communicantes of the upper dorsal nerves. What is the respective origin of these nerves in the heart is not as yet certain, but the recent investigations of Köster[1] indicate that the depressor nerve, the afferent nerve of the heart, *par excellence*, is in reality the sensory nerve of the aorta and not of the heart itself. The centres, both of the cardiac inhibitors and accelerators and augmentors are situated in the medulla oblongata—not in the cerebellum, as Willis supposed—the former in the vagal nucleus, where a pin-prick will arrest the heart, the latter in a point not yet accurately determined.

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[1] *Ueber die Ursprung des N. Depressor*, *Neurologisches Centralblatt*, 1901, p. 1032.

These cardiac centres are in a more or less continuous state of so-called automatic activity, which is capable of being exalted, depressed, or otherwise modified by impulses proceeding from the heart itself and blood-vessels, from the somatic and splanchnic periphery as well as from the higher regions of thought and feeling.

We have, as a rule, no direct voluntary control over the heart's action—perhaps a wise provision of Nature against the temptation to try foolish and dangerous experiments. Cases, however, are on record in which, apparently hy voluntary effort, the action of the heart has been inhibited or rendered almost imperceptible. The best authenticated instance of this kind is that of Colonel Townsend (related by Cheyne in his "English Malady," 1733), who was able at will to throw himself into such a state of suspended animation that his heart and breathing ceased to be appreciable by ordinary tests. More wonderful things have been related of the Indian fakirs. We do not as yet know the real mechanism of this voluntary hybernation, but Ed. Weber proved on himself that the action of the heart can be reduced *ad* *deliquium animi* by voluntary compression of the chest and forcible expiration with the glottis closed. This exerts such pressure on the great veins as completely to obstruct the flow of blood through the heart and to cause it to stop for several beats. Some of the cases of apparently direct voluntary control over the heart's action may be, therefore, accounted for through the medium of the respiratory mechanism; but there are others[1] in which the rate of the heart has been capable of acceleration at will, independently of the respiration and without calling up special ideas. In all such instances there has been also unusual power over the voluntary muscles.

Like the heart, the calibre of the blood-vessels is regulated by mutually antagonistic sets of nerves—the vaso-constrictors (vaso-motor) and the vaso-dilators (vaso-inhibitory). That the lumen of the blood-vessels was subject to variation under the influence of the nervous system was postulated long before it was actually demonstrated. The credit of this prevision has been assigned to various writers.

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[1] Tarchanoff, "Pflüger's Archiv.," Bd. xxxv., 1884; also Pease, *Boston Med. and Surg. Journal*, May, 1889.

Harvey, as we have seen, intended to discuss the subject in his "Medical Observations." The philosopher Malebranche[1] is said to have propounded a vaso-motor theory of emotional expression according to which the flow of vital spirits to the brain and the organs was regulated by a special system of nerves. But Willis appears to have first actually demonstrated the nerves of the blood-vessels and to have indicated their true function ; for he describes nerves from the cardiac and abdominal plexuses as accompanying the blood-vessels, which he graphically likens to twigs of ivy embracing and surrounding their trunks and branches. Of their function, in speaking of the solar plexus, he says :—

> "That from this plexus many fibres and shoots going forth are inserted into the Trunk of the Aorta nigh its descending, and that these reaching towards the intestines accompany the Blood-carrying vessels, and in several places climb over them ; from hence it may be inferred that nerves also in the Abdomen are like Bridles and Reins cast on the sanguiferous Vessels, which, either by straining or pulling them together, may

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[1] Quoted by Lange, *Ueber Gemüthsbewegungen*, 1887.

> sometimes retard, sometimes incite the course of the blood according to the needs of the lower Viscera."[1]

The tightening or relaxation of the grip of the vaso-motor nerves on the blood-vessels, imagined by Willis, was shown by the discovery of Henle, in 1840, of the muscular nature of the middle coat of the arteries to be a contraction or relaxation of the walls of the blood-vessels themselves. But the actual proof of the action of the vaso-motor nerves was first given in 1851 by Claude Bernard,[2] in the well-known dilatation of the vessels of the rabbit's ear on section of the cervical sympathetic, followed almost immediately by the demonstration of the converse results on electrical stimulation of the distal end of the cut nerve by Bernard himself, Brown-Séquard,[3] and Waller.[4] Similar phenomena were speedily proved to obtain in reference to the vessels of the abdomen, the limbs, and most of the organs of the body. The blood-vessels are normally in a state of tone or semi-contrac-

[1] Chapter xxvii., p. 138.

[2] Comp. Rend. Biol., 1851, p. 163.

[3] *Philadelph. Med. Examiner*, Aug., 1852.

[4] Comp. Rend., 1853, p. 378.

tion, which is kept up mainly by the nervous system. The centre which governs the calibre of the blood-vessels is situated in the pons-medulla, probably in the group of cells known as the antero-lateral nucleus—the homologue of the cells of the intermedio-lateral tract of the spinal cord (Gaskell). Hence on section of the cord below this point there is extensive vaso-dilatation, with an enormous fall in the blood pressure. But though this is the chief centre of vaso-motor regulation, there is evidence that there are also subsidiary centres in the cord itself. For, after section below the medulla oblongata, the vessels after a time regain a considerable amount of tone, and reflex alterations in their calibre can be elicited by appropriate stimuli. The blood-vessels also possess an intrinsic activity similar to that of the heart itself, and exhibit slow rhythmical variations in calibre; and the recent experiments of Bayliss[1] prove that they respond to mechanical variations in internal pressure—constricting or relaxing, with a rise or fall respectively. The antagonistic nerves, the vaso-dilators, the homologues of the cardiac inhibitors, indicated by Schiff, were first clearly demonstrated by Bernard[1] in 1858, in the chorda tympani. (Electrical irritation of the branch of this nerve distributed to the submaxillary gland was found to cause such a degree of vascular dilatation that the blood flowed from the veins in jets and arterial in hue—a condition entirely independent of the function of the gland as such, inasmuch as it occurs with the same distinctness when the function of the salivary gland has been paralysed by atropine. The phenomenon is a pure instance of vaso-dilatation.)

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[1] *Journal of Physiology*, xxviii., p. 220, 1902.

Bernard's discovery of the vaso-dilators of the chorda tympani was followed in 1863 by that of Eckhard[2] of the vaso-dilator action of the nervi erigentes, and the existence of a special set of vaso-dilators running in separate channels from the constrictors was thus conclusively established. Subsequent researches have shown that vaso-dilators exist also in other nerves, which at first sight appear to contain only vaso-constrictors. Their presence, however, is only masked by the greater number and greater excitability of the constrictors, but when special methods of stimulation are employed, the action of the vaso-dilators is made clearly manifest. All the organs supplied with vaso-constrictors are furnished also with vaso-dilators, but the proportion differs, and in some organs, such as the brain and lungs, it is still a question whether there are any vaso-motor nerves at all. In reference to the lungs, in which Bradford and Dean,[1] as well as François Franck,[2] claim to have demonstrated the existence of vaso-motor nerves, the recent experiments of Brodie and Dixon[3] are of special importance and significance. They found that no change in the calibre of the pulmonary arterioles could be produced by direct stimulation of any of the fibres proceeding to, or emanating from, the stellate ganglion. Further, when the lungs were perfused with blood containing suprarenal extract, which, as has been shown by Schäfer and Oliver,[4] usually causes constriction of the blood-vessels, no such results occurred in the pulmonary blood-vessels but rather the reverse. This led to the discovery that when the arterioles of organs, otherwise occluded by suprarenal extract, have had their vaso-motor nerves previously paralysed by apocodeine or cocaine, perfusion with suprarenal extract causes dilatation instead of constriction. That this result is not due to paralysis of the muscles of the arterioles is proved by perfusion with chloride of barium, which causes intense constriction and complete stoppage of the outflow. These experiments show, therefore, that suprarenal extract acts by stimulating the nerve-endings of vaso-constrictor nerves, and that there is no evidence of such in the pulmonary blood-vessels.

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[1] *Journ. de Physiologie*, i., pp. 237 and 649.

[2] Beitr. 3. Anat. u. Physiol., iii., p. 123.

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[1] *Journal of Physiology*, 1894, vol. xvi., p. 34.

[2] *Archives de Physiologie*, xxvii., pp. 744 and 816, 1895.

[3] Brodie: "Arris and Gale Lectures on the Pulmonary Circulation," *The Lancet*, March 22, 1902, p. 803.

[4] *Journal of Physiology*, vol. xviii., p. 230, 1895.

Generally, it may be said that those organs in which it is necessary for occasional purposes to have an active flow of blood, such as the salivary glands, kidney, and generative organs, are specially provided with vaso-dilators, which sometimes run in separate channels. The centres of the vaso-dilator nerves have not been determined with the same precision as those of the constrictors. It is probable that they are related to the segments of the cord, from which they respectively arise. This at least is true of the nervi erigentes, for when the cord is divided above their origin their function can be excited or inhibited reflexly just as in normal conditions (Goltz). Vaso-dilatation is not, however, always an active effect, but sometimes only a cessation of normal tone; and there is one afferent nerve, the action of which is always vaso-dilatation. This —the depressor, discovered by Ludwig and Cyon in 1866—plays a very important *rôle* in the regulation of the circulation. It is one of the afferent or sensory nerves of the heart, arising either in the heart itself, or, according to the researches of Köster mentioned above, at the base of the aorta. Stimulation of this nerve, arising normally from states of tension, inhibits the action of the vaso-motor centre and causes intense dilatation, more particularly of the abdominal system of blood-vessels. It is by the interdependence of the cardiac and vaso-motor nerve mechanisms, and the manifold ento- and epiperipherical stimuli by which they are influenced, that the heart is protected from undue strain; that great and prolonged variations in the differential pressure between the arteries and veins are obviated or compensated; and that each organ obtains its due measure of blood in accordance with the calls made upon it. We cannot doubt that the cardiac and vascular reflexes are essentially of a protective nature, like reflex actions in general. But though the teleological significance of many of these is obvious, the usefulness of others is still involved in obscurity. We can readily understand why, as Marey has beautifully demonstrated, the heart should be inhibited when the resistance in front is too great for it to contend with safely; and why, under similar conditions, impulses should be conveyed by the depressor nerve, which diminish the resistance by dilating the great vascular area of the abdomen. In this we have an instance of the protective action of the vascular and cardiac reflexes on the heart itself. On the other hand, the vagus may be called into play with the view of protecting other organs, especially the brain, from undue pressure and congestion. Great rise of intracranial pressure causes slowing of the heart. This is brought about by direct action on the medulla itself. For, as Franck has shown, when the brain is isolated from the rest of the body, with the exception of one vagus, injection of blood into the carotid causes the heart to beat faster or slower according to the degree of pressure exerted. And it has been argued by Roy and Adami[1] that in this we have a protective against cerebral congestion, inasmuch as the slowing of the heart from increased intracranial tension is brought about by action on the medullary centres and not from within the heart itself. The protective mechanism of cardiac acceleration is exemplified in the increased rapidity of the heart, which is excited by conditions which tend to lower the pressure in the bulbar centres—such as change from the horizontal to the vertical posture, and under all conditions which lead to dilatation of any large vascular area.

All organs in a state of activity are able, either by local action on the blood-vessels or by reflex vaso-dilatation, to secure an increased flow of blood. If the vascular area thus dilated is at all extensive the lowering of blood pressure, which would be thus induced, is counteracted by greater rapidity of the heart, and constriction of the vessels in other regions. Thus dilatation of the abdominal vessels during the act of digestion is accompanied by the proverbial coldness of the skin, and the constricted vessels and cold feet of active brain work are familiar to us all. These instances of the obviously protective action of the cardiac and vascular reflexes might be multiplied indefinitely, but there are many others the significance of which is not so apparent. Among these may be mentioned the reflex inhibition of the heart, which is so easily excited by irritation of the air passages[1] and lungs.[2] There is little reason to doubt, however, that like the similar inhibition of the respiration from irritation of the interior of the heart,[3] they play an important part in the mutual regulation of the intimately interdependent functions of the circulation and respiration. The cardiac, vaso-motor, and respiratory centres are all closely inter-related to each other. Of these the respiratory is most readily influenced, both from the periphery and the higher centres, and it is not improbable that many of the variations in cardiac rhythm and vascular tone so induced are in reality conditioned only mediately through the respiration. With the exception of the depressor and the nerves of the air passages and lungs, moderate stimulation of perhaps all other sensory nerves, general and special, causes constriction of the blood-vessels, with a rise of blood pressure and acceleration of the heart. This, however, is a rule liable to many exceptions, the conditions of which are not as yet in all respects satisfactorily determined.

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[1] *Philosophical Transactions*, B. 183, p. 251, 1893.

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[1] Dogiel, Holmgren.

[2] Brodie and Russell : *Journal of Physiology*, vol. xxvi., p. 92, 1900.

[3] Franck : *Travaux du Laboratoire de Marey*, 1880.

In states of exhaustion, and when the stimulation is of a sudden or intense character, more particularly when of visceral origin, there is a fall in blood pressure and reflex inhibition of the heart, which, in certain conditions, may be fatal. As an instance of this kind one may quote Goltz's famous "Klopfversuch," by which it is shown that a smart tap on the abdomen of a frog causes stoppage of the heart and dilatation of the splanchnic blood-vessels. The vascular dilatation so induced is so enormous that practically the whole of the blood in the body is accumulated in the abdomen, and the animal dies by hæmorrhage into its own veins. The heart may continue to beat, but to no purpose ; its cavities are empty and no blood passes through it. It is, in all probability, to vaso-motor paralysis of this nature that the symptoms of surgical shock are due, as has been maintained by Lauder Brunton,[1] Crile,[2] and others. In this relation also the recent researches of Embley[3] on the cause of death from chloroform are deserving of the most attentive consideration. In the early stages of chloroform inhalation the heart is weakened and more susceptible to the influence of the vagus. The inhibitory mechanism is also more excitable, so that there is thus a twofold risk of permanent inhibition of the heart, especially when the blood pressure is greatly reduced. Fortunately, in profounder anæsthesia the cardiac reflexes are almost abolished, so that in addition to its beneficent action in annulling pain, chloroform minimises the otherwise dangerous, or even fatal, depression that might result from severe surgical procedure.

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[1] "Syncope and Shock," 1873.

[2] "Surgical Shock," 1897.

[3] *British Medical Journal*, April, 1902.

Most, if not all, of the cardiac and vascular reflexes are capable of being elicited through the medullary centres alone, and they can be obtained with great facility, as Brodie and I have found, in decerebrate animals.

The cardiac and vascular reflexes have an especial bearing on the circulation in the brain. The cerebral circulation has many features of an exceptional character. The doctrine propounded by Monro Secundus, supported by Kellie and Abercrombie, and most ably advocated at the present day by Leonard Hill,[1] is that, as the cranium is a closed cavity and the brain substance incompressible, the quantity of blood within the skull must practically be at all times the same—the blood flowing out of the veins to make room for that flowing in by the arteries—those conditions only being excepted in which fluid or other matter is effused or secreted by the blood-vessels, for, in such circumstances, a quantity of blood equal in bulk to the effused matter will be pressed out of the cranial cavity. It has been argued in opposition to this doctrine that the free ebb and flow of the cerebro-spinal fluid between the cerebral and spinal cavities must allow of considerable variation in the volume of blood in the cranium, but under normal conditions the amount of this fluid is so small as to be practically a negligible quantity. The variations in blood-supply extend for the most part only to the relative proportion between the arterial and venous. The brain fills the cranial cavity as a hand a glove, and is closely appressed to the interior of the skull-cap. The pressure exerted from within outwards varies greatly; directly with the venous, and only proportionately with the arterial pressure. It is increased with each pulsation of the heart, and with each expiration if at all forcible. The average is about 100 millimetres H_2O, but the pressure is of importance purely in its bearing on the circulation. The functions of the brain cease if the arterial pressure is too low to cause an effective flow throughout the capillaries, or if the venous pressure is too high to permit of the arteries emptying themselves. In either case the circulation in the brain fails, and loss of consciousness ensues. The brain tissue, however, as such can carry on its functions at any pressure from zero up to 50 millimetres of Hg. or more. Thus, in a patient who had been trephined, and the brain, therefore, exposed to atmospheric pressure, Hill found that the intracranial pressure fell slightly below zero when he stood upright; and in a case of strychnine convulsions the pressure was estimated at 50 millimetres Hg., and yet both patients retained their mental faculties unimpaired. Up to the present no satisfactory experimental evidence has been furnished as to the existence of vaso-constrictors or vaso-dilators of the cerebral blood-vessels. Morison,[1] however, and Gulland[2] have both, within the last few years, demonstrated the presence of nerve plexuses on the vessels of the pia mater in all respects like those of vaso-motor nerves elsewhere. Yet no active change in the calibre of the cerebral blood-vessels can be produced by stimulation of the cervical sympathetic or stellate ganglion

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[1] "The Physiology of the Cerebral Circulation," 1896.

[1] *Edinburgh Medical Journal*, 1898.

[2] *British Medical Journal*, vol. ii., 1898, p. 78.

—practically the whole of the sympathetic supply to the carotid and vertebral arteries—or even, according to Hill, of the central end of the cut spinal cord or the vaso-motor centre itself. The opposite results obtained by Nothnagel, Cavazzani, and others are attributable to defective methods of experiment, which do not differentiate between active and passive variations in the calibre of the vessels, or exclude changes due to mere atmospheric exposure. This negation of vaso-motor regulation of the cerebral blood-vessels seems to be in flagrant contradiction with the positive demonstration of nerves accompanying the vessels of the pia mater, as well as with other facts, such as the apparently independent variations of the plethysmographic volume records described by Mosso, and we can scarcely doubt that there must be some intrinsic mechanism which can secure a greater flush of blood in one part as compared with another in an organ in which there is proved localisation of function. Roy and Sherrington[1] believed that they had discovered such a mechanism in the products of cerebral metabolism dissolved in the lymph which bathes the cerebral arterioles—products capable of inducing variation in the calibre of the blood-vessels in correspondence with local variations in functional activity. This, however, has been contested by Hill. Brodie and I have, however, succeeded in rendering it more than probable that the cerebral blood-vessels are under the influence of vaso-motor nerves, though so far we have not been able to determine their origin and course.

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[1] *Journal of Physiology*, xi., p. 103, 1890.

As already mentioned, perfusion with suprarenal extract of an organ supplied with vaso-motor nerves causes constriction of the arterioles so that the flow through the capillaries is diminished or altogether stopped. It was found by Biedl and Reiner[1] that injection of suprarenal extract into the cerebral end of the carotid caused a rise of blood pressure in the circle of Willis, and diminution of the outflow from one of the lateral sinuses—a result which might be attributed to the action of adrenalin on the terminals of cerebral vaso-motor nerves. But the conclusion is not free from fallacy owing to the non-determination of the conditions affecting the outflow from the other sinus. This fallacy, however, is eliminated by Brodie's method. By the injection of adrenalin into the basilar artery of the carefully removed brain, and measurement of the outflow from the torn sinuses, he found that with a moderate amount there was distinct diminution of the outflow, and complete stoppage when the dose of the extract was increased. This appears to afford satisfactory proof of the existence of cerebral vaso-constrictors, though, as compared with those of other organs, their influence is relatively slight; and there seems, therefore, no further reason for doubting the existence of some intrinsic vaso-motor regulation of the cerebral circulation. The cerebral circulation, however, varies for the most part only passively with the circulation as a whole. The blood is so disposed in the body that it may be temporarily diverted from one region in order to secure a richer supply to another which requires it. It has been calculated that when the body is at rest the thoracic and abdominal organs contain from 60 to 70 per cent. of the whole blood. In the state of activity this percentage is reversed. Thus Ranke estimates that in the state of rest the neuro-muscular apparatus contains on the average only about 36 per cent. of the blood, while in the state of activity the percentage is nearly doubled. The rapidity of the circulation in the brain in a state of activity is greater than in the state of quiescence or sleep. This is brought about by the constriction of the splanchnic and cutaneous blood-vessels through the agency of the vaso-motor centre. In illustration of this an ingenious apparatus has been devised by Mosso. When a person lies flat on a couch swinging on a horizontal axis, with the head and feet accurately counterpoised, the head goes down when the brain is at work; on the other hand, the feet sink when the individual falls asleep, indicating that the blood has returned to the extremities. In tranquil sleep a sound or any kind of sensory stimulus suffices to reverse the position of the head and feet, and that, too, when the stimulus has not been sufficient to cause the sleeper to awake or to retain any knowledge of the event. Every sensory stimulus of moderate intensity has the effect of raising the blood pressure by constriction of the cutaneous and splanchnic areas. We may see in this a protective mechanism whereby impressions on the organs of sense not only awaken perception but provide the centres and apparatus of thought and volition with the means of energising in accordance with the needs of the occasion. The sensory nerves and vaso-motor centre therefore act, in the figurative language of Mosso, as "sentinels on the defensive, watching continuously, and sounding the alarm when danger is nigh."[1]

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[1] *Pflüger's Archiv*, Band lxxix., p. 158, 1900.

The circulation in the brain is largely dependent on the tone maintained by the vaso-motor centre. Conditions which materially lower the vascular tone, such as prostrating diseases, certain toxic agents, and the like, tend to induce cerebral anæmia and impairment of cerebral energy, especially in the upright posture. For though under normal conditions the influence of gravity is not appreciably felt, it becomes very apparent when the vascular tone is defective. Hence in such conditions syncope readily occurs when the individual is suddenly raised from the horizontal to the vertical position, or obviated when the positions are reversed. In a less degree the failure of the cerebral circulation from defective blood pressure is seen in the heaviness and drowsiness which follow a heavy meal, with its consequent dilatation of the abdominal blood-vessels. Some individuals cannot do active brain work in the upright posture, and instinctively adopt such attitudes as favour the flow of blood to the head. The girding of the loins for active effort has its philosophy, according to Roy and Adami,[1] in the compression of the abdominal vessels, which raises the blood pressure and increases the output of the heart.

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[1] "Fear," p. 123, 1896.

The influence of the blood pressure on the circulation of the brain naturally leads to a consideration of the influence of cerebral activity, more particularly states of feeling, on the heart and blood-vessels. This is a highly complex problem, and though it has actively engaged the attention of many eminent physiologists and psychologists, the results arrived at are neither altogether definite nor harmonious. The influence of states of feeling on the circulation is, however, so patent that at all times and in all languages they have been expressed in terms of the heart. The very obvious display of emotional states in this manner has led James[1] and Lange,[2] particularly the latter, to regard the vaso-motor reactions and the conditions thus secondarily induced in the viscera as the essential basis of the vivid or so-called "courser" emotions, such as joy, sorrow, fear, and anger. There is much to be said in favour of the view that the organic sensations, primary or secondary to vascular changes, constitute an important factor of emotional states, but there are serious objections to regarding them as their essence. The sensations and emotions have their affective tone as such independently of the vascular concomitants, and it has been contended that the vascular reactions occur in point of time subsequently to the actual manifestation of the feeling in consciousness. Sherrington's[3] experiments have also an important bearing in this relation. When in dogs the spinal cord had been severed headward of all the sympathetic nerves of the thoracic, abdominal, and pelvic viscera—thus separating the brain practically from all connection with these viscera, from the skin of the trunk and limbs, and the blood-vessels from the vaso-motor centre—yet these animals under appropriate stimuli exhibited indubitable signs and gestures expressive of pleasure, anger, fear, disgust, and the like. The same phenomena were observed even when in addition the vago-sympathetic nerves were divided, thereby rendering insensitive also the stomach, lungs, and heart itself. Unless we can assume with Ribot[1] that there is a *mémoire affective*, and with Dewey[2] that there is a revival in idea of the organic states that have in past experience been habitually associated with particular feelings, we must admit from these experiments that, apart from all vascular and visceral effects, emotions and passions are capable of being felt and expressed, at least in the domain of the muscles of animal life, with all their appropriate and characteristic gestures. We can scarcely doubt, however, with James that such eviscerated and disembowelled emotions can only have been a mere simulacrum of those which would otherwise have thrilled throughout the whole frame of the animal. But even if we thoroughly recognise the immense importance of the visceral factor in emotion, it would be rash, if not altogether erroneous, to regard this, with Lange, as secondary only to vasomotor changes. For there are many facts—notably the novel and luminous experiments of Pawlow[1] on secretion—which prove that psychical states influence the processes of secretion not mediately through the circulation but directly through the secretory nerves of the glands. The most manifest organic expressions of emotion, however, are those in the domain of the circulation with which, for the present, we are more immediately concerned.

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[1] "Waist Belts and Stays," *National Review*, November, 1888.

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[1] "Principles of Psychology."

[2] *Ueber Gemüthsbewegungen*, 1887.

[3] "Proceedings of the Royal Society," May 10, 1900.

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[1] *Psychologie des Sentiments*, 1899.

[2] *Psychological Review*, 1895.

The observations of Binet and Courtier[2] show that every feeling, whether agreeable or painful, acts primarily as an excitant. The passage from the state of repose into the state of activity, intellectual or emotional, causes vaso-constriction. The stronger the stimulus the greater the effect, and for this reason, perhaps, the more painful feelings and emotions, which can be more readily experimentally induced, cause more marked vaso-constriction than those that are more agreeable. The heart is accelerated, the respiratory rhythm is altered, becoming more rapid and more profound, with obliteration of the respiratory pause, and at the same time there is a general rise of blood pressure.[1] If the volume of the brain is simultaneously registered by the plethysmograph, as Mosso has described, and Brodie and I have verified, one observes almost constantly an increase. This seems to indicate that there is a relation of antagonism between the volume of the brain and that of the extremities. But this is not an absolute rule, for Mosso has observed that the oscillations in the brain volume do not in all respects run parallel to those of the extremities, probably owing to local variations in the cerebral blood-vessels themselves. Whether the quality of the sensation or emotion as such—*i.e.*, whether it be agreeable or disagreeable, pleasurable or painful—is always associated with characteristic and uniform changes in the circulation is still the subject of considerable difference of opinion. It is probable that the discrepancies are largely occasioned by the fact that the characters of feelings are, as maintained by Wundt, much more complex than can be expressed in simple terms of pleasure or pain. For besides being pleasurable or the reverse (Lust—Unlust), feelings are exciting or soothing (Erregung—Beruhigung), straining or relaxing (Spannung—Lösung), and vary in intensity. And Brahm holds[1] that each of these conditions has its specific influence on the circulation, hence the results may so vary in different individuals that absolute uniformity probably does not exist. Apart from the suddenness of the change from a state of repose to a state of activity, whether this be indifferent, such as mere surprise, or of a painful or pleasurable character, the balance of evidence is in favour of the view that pleasurable sensations and emotions are accompanied by vascular dilatation and low tension, while the contrary are associated with vascular constriction and high-tension pulse. This is borne out, not only by experiments on normal individuals,[1] but by observations on the morbid states of joy and sadness in the typical forms of insanity.[2]

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[1] *Le Travail des Glandes Digestives*, 1901.

[2] *La Vie Emotionelle*, *L'Année Psychologique*, 1897.

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[1] Binet and Vaschide, *ibid.*

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[1] *Experimentelle Beiträge zur Gefühlslehre*, *Philosophische Studien*, Band xviii., Heft i., 1901.

The vascular dilatation of pleasurable states of mind signifies also a more active circulation and exaltation of all the vital processes—dynamic and metabolic—while the opposite condition obtains in states of mental pain. Conversely, also, given the vascular and corporeal state arising spontaneously or induced artificially, usually associated with any particular mood, trains of thought and states of feeling corresponding therewith are apt to arise in consciousness. This, in all probability, as Lange indicates, is the origin of the use of stimulants and nervines of various kinds, which all nations and at all times have discovered for themselves. That "wine maketh merry the heart of man" is only a more picturesque statement of the truth I am endeavouring to convey in more didactic style. Pleasurable and painful emotions are thus not merely subjective states of consciousness, but at the same time objective corporeal conditions of exalted or depressed vital energy respectively, which manifest themselves not only in outward attitude and gestures but in the relative power of the organism to withstand debilitating agencies of all kinds. To promote the one and to combat the other is therefore to the physician not less important—and perhaps more a mark of therapeutic skill—than a judicious selection from the materia medica. There is thus a similarity, if not an identity, between the effects on the circulation of painful and pleasurable sensations and painful and pleasurable emotions, and at bottom they are probably based on the same physical substrata. Pain and pleasure are merely the subjective aspects of physiological conditions harmful or beneficial to the organism — a harmonious relation or the reverse between the processes of integration and disintegration.

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[1] Lehmann: *Die Körperlichen Äusserungen psychischer Zustände*, 1899.

[2] On this I might quote the observations of Dumas: *La Tristesse et la Joie*, 1900; Broadbent: "The Pulse," 1890; Maurice Craig: "Blood Pressure in the Insane," *The Lancet*, June 25, 1898; Dawson: "The Rôle of the Blood-supply in Mental Pleasure and Pain," *Journal of Mental Science*, February, 1900; and others.

As all the emotions, however complex, are founded ultimately on the affective tone accompanying the exercise of the organs of sensation in all their relations, visceral or somatic, the emotional and sensory substrata are one and the same. And it is not improbable that each organ has its own affective tone, its own centre, and contributes its own share to the general emotional result. Whether the feelings are conditioned from within or without, by presentation or representation, the effect on the circulation is the same. That which seems to constitute the chief difference between simple feelings and emotions is the relatively greater influence of the latter as compared with the former. Emotions are unitary composites of simple feelings and the resulting aggregate is much more powerful than any one of its individual elements. Fear, which is mental pain, causes greater acceleration of the heart, greater vascular constriction, and greater pallor than can be induced by mere physical pain. Not only is the circulation profoundly affected but there is a tendency to irradiation beyond the cardiac and vaso-motor into all the splanchnic functions, so that we observe *inter* *alia* profuse perspiration, horripilation, and movements of the abdominal and pelvic viscera, which can rarely, if ever, be reflexly induced by any degree of peripheral stimulation. In its more intense forms fear may for ever arrest the heart-beat or so break down all the protective mechanism of the organism that flight, defence, or other means of adaptation are rendered impossible, and the animal falls an easy victim to the danger assailing it.

When we inquire by what mechanism the centres of thought and feeling influence the heart and blood-vessels—whether there are cortical cardiac and vaso-motor centres properly so-called, or whether the cerebral hemispheres act only indirectly upon the cardiac and vaso-motor centres of the medulla oblongata, we come upon a subject on which, in the present state of our knowledge, it is necessary to speak with caution, avoiding too confident or dogmatic assertion. Several observers[1] have described variations in the rate of the heart and tone of the blood-vessels as resulting from irritation of various portions of the cerebral hemisphere, particularly the motor area or adjoining region. But the careful experiments and weighty considerations advanced by François Franck[1] render it, I think, probable that all the cardiac and vascular effects that occur are merely the correlated concomitants of the functional activity of the motor centres, excited in a more or less obvious epileptic manner ; or that the cerebral hemispheres are only the point of departure of stimuli which influence the cardiac and vaso-motor centres of the medulla oblongata in precisely the same way as those which originate in the periphery. The results are inconstant—sometimes acceleration, sometimes inhibition ; they cannot be foretold ; they do not vary with the point of stimulation ; they have none of the features of the contra-lateral manifestations so characteristic of stimulation of the motor centres ; and there is no good ground for assuming that there are any definite areas controlling respectively acceleration, inhibition, vaso-constriction, or vaso-dilatation. It is, indeed, probable that many of the reactions on the heart and blood-vessels which have been described as resulting from cortical excitation are only mediately produced through the respiratory centre, which we know is to a considerable extent governed from the cerebrum. The cardiac and vascular centres in the proper sense of the term are in the medulla oblongata and the hemispheres are to them as much peripheral as the sensory nerves in general. The same may perhaps be affirmed of the so-called centres of the salivary and digestive glands recently described by Bechterew and his pupils.[1]

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[1] Schiff, Bochefontaine, Danilewsky, and others.

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[1] *Les Fonctions Motrices du Cerveau*, 1887.

Through what centrifugal channels the cerebral hemispheres influence the cardiac and vaso-motor centres is not definitely ascertained, but they probably traverse the tegmentum beneath the corpora quadrigemina. For in this region electric stimulation, as Danilewsky, Lauder Brunton, myself, and others have shown, invariably produces such alterations of cardiac and respiratory rhythm and vascular tone as might well be regarded as signs of irritation of the paths by which the cortical centres transmit their influence to those of the medulla oblongata. We can, however, scarcely expect by artificial methods to reproduce the conditions underlying any particular emotion, and no one has yet succeeded in imitating, either by reflex or central stimulation, the blush so characteristic of the emotions of modesty or shame. But further to discuss these and kindred topics would lead me far beyond the limits of an occasion such as this.

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[1] *Die Corticalen Secretorischen Centra der Verdauungsdrüsen*, *Archiv für Anatomie und Physiologie*, *Physiologische Abtheilung*, Hefte iii. and iv., 1902.

My object has been to present to you a concise sketch of some of the principal relations of the heart to the nervous system which we have learnt since the time of the immortal Harvey. I have left many problems untouched, and as to those on which I have ventured I have, I fear, succeeded only in indicating how much is still hypothetical and uncertain. Doubtless some Harveian orator of the future, if he takes up the same theme, will be able to expound it more luminously in the light of fuller and riper knowledge. This will surely come if only we follow in the footsteps of our great master, and obey his wise injunction to "search out Nature by way of experiment."

Abstract of Three Lectures

ON

THE PULMONARY CIRCULATION

MORE PARTICULARLY IN RELATIONSHIP TO VARIATIONS IN CARDIAC ACTIVITY

Delivered before the Royal College of Surgeons of England on February 17th, 19th, and 21st, 1902.

BY

T. G. BRODIE, M.D. LOND.

ARRIS AND GALE LECTURER; DIRECTOR OF THE CONJOINT LABORATORIES
OF THE ROYAL COLLEGE OF PHYSICIANS OF LONDON AND OF THE ROYAL
COLLEGE OF SURGEONS OF ENGLAND; AND LECTURER ON
PHYSIOLOGY AT THE LONDON SCHOOL OF
MEDICINE FOR WOMEN.

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ABSTRACT OF

Three Lectures

ON

THE PULMONARY CIRCULATION, MORE PARTICULARLY IN RELATIONSHIP TO VARIATIONS IN CARDIAC ACTIVITY.

PREVIOUS workers upon the circulation through the lungs have mainly confined their attention to a study of the pressure changes in a pulmonary artery or vein in comparison with the pressure records in the aorta, and from these operations they have drawn conclusions as to the general variations of the circulation through the lungs. The close relationship of the two sides of the heart to the pulmonary vessels renders it, in most cases, a matter of considerable difficulty to decide, from observations of pressure changes alone, whether or not any active change has taken place in the pulmonary vessels. Hence the observations forming the subject-matter of the main portion of these lectures have been conducted by Dr. W. E. Dixon and myself during the past two years and took their origin from the application of another method—the plethysmographic—to the lung. This method has already proved of very great value in the study of the circulation in organs on the systemic circulation and has also proved a most valuable means of studying the circulation through the lungs under various conditions.

The operation consists in first exposing the lower lobe of the lung by the resection of two or three ribs. The lobe is then thoroughly freed up to the root of the lung, and if the object is to study the vascular changes one of two methods is adopted. In our first experiments we prevented the entrance of air into the lung by gently compressing it before inserting it in the oncometer. In this way the lobe does not expand with the ordinary air pressure used in artificial respiration. In a second series of experiments we first distended the lobe to its normal extent and then plugged the bronchus leading to the lobe by a pledget of cotton-wool soaked in vaseline, inserted by means of a long probe passed down the trachea The lobe was then fixed in the oncometer and its variations in capacity recorded by connecting the oncometer to a piston or bellows-recorder. We have always simultaneously registered the aortic pressure by a mercury manometer. The advantages of the method lie chiefly in that it gives us at once a record of the changes in amount of blood contained within the lungs, thus showing immediately the production of an anæmia or congestion of the lung capillaries. By combining the information thus gained with the pressure changes in the four chambers of the heart we can often definitely determine the nature of the change that has been produced in a more certain way than can ever be obtained from records of the pressure changes only.

Variations in the flow of blood through the lungs may be caused by changes in the activity of the right heart or in the resistance offered by the pulmonary vessels and capillaries, and to this extent the problems offered for our study are similar to those so fully studied in the systemic circulation. Whereas, however, variations in the activity of the right heart produce but small effects upon the flow through the systemic capillaries, unless those changes be very pronounced, variations in the working of the left heart produce at once marked changes in the blood contents of the lungs. As Stolnikow has pointed out, the liver acts to a considerable extent as a blood reservoir on the systemic circulation, so that the right heart may take a greater or less volume of blood from the great veins without materially modifying the systemic pressure. So the lungs play a similar part to the left heart, acting as a reservoir in which blood may be stored if the left heart for some reason diminishes its output, and conversely contains a reserve of blood upon which the left heart draws when its output increases. It is this very close relationship to the two sides of the heart which makes the study of the pulmonary circulation of such difficulty and has led many workers into considerable errors.

In these lectures we shall therefore discuss the various experiments which we have performed in the following order: (1) the alterations in the pulmonary circulation due to purely cardiac changes; (2) the alterations due to variations in the systemic circulation, which may effect changes by alterations in the output of the left heart or by modification in the flow to the right heart; and (3) an examination of any changes due to alterations in the resistance offered by the pulmonary vessels.

The blood-volume curve given by a lobe of the lung in which the bronchus has been plugged in the manner described above gives the following features. There is a small increase in volume at each inflation by the artificial respiration apparatus of the remaining lobes of the two lungs, and there is a very marked volume-pulse occurring with each heart-beat. This volume-pulse is systolic in time and is therefore due to the output from the right heart.

Turning in the first instance to the effect of alterations in cardiac activity upon the pulmonary circulation we may first of all discuss the changes caused by excitation of the augmentors and accelerators on the one hand and of the inhibitory fibres on the other. Stimulation of the accelerator fibres which also usually causes augmentation, as a rule, leads to an increase in the volume of the lung and a rise in the aortic pressure. The increased volume of the lung usually persists longer than the rise in aortic pressure. In a few instances no increase in lung-volume is recorded and these cases are usually accompanied by a more marked rise in aortic pressure. Simultaneous records of the blood-pressure in the pulmonary artery and aorta show a marked rise in the former which may or may not be accompanied by a rise in aortic pressure. From these results we conclude that the nerves chiefly affect the right side. The output from this side usually shows a greater increase than that from the left: hence the blood-volume of the lung is increased. The various effects upon the aortic pressure show how variable the action is upon the left heart.

Stimulation of the vagus also gives very interesting results. With weak stimulation, resulting in much slower beat, the aortic pressure falls gradually and a marked decrease in the volume of the lung is recorded. A stronger stimulation, resulting in a much more marked fall in aortic pressure, leads to a quicker decrease in the lung-volume which, however, is no more extensive. In these cases the right auricle pressure rises considerably, but the effect upon the left auricle is much less marked. Hence we must conclude that the action of the vagus upon the heart is more particularly felt upon the right side. The diminution in volume of the lung can only mean a larger diminution in output of the right ventricle as compared to that of the left.

The next series of changes to be studied are those due primarily to an alteration in the systemic circulation. Of these we must distinguish two groups—namely, those in which respectively a rise or a fall in the aortic pressure is produced. These may exert an effect upon the pulmonary circulation either through a change in the rate of flow of blood into the right auricle or by affecting the output from the left heart. At present the latter of these two factors has been chiefly studied.

As the simplest case we may take the effect of restricting the outflow from the aorta by partially or completely blocking the aorta at the lower end of the thorax. Waller and later observers have shown that the result of clamping the aorta is to cause a big rise in the aortic pressure, followed quickly by one in the left auricle. There is no rise in the pulmonary artery pressure unless the compression is kept up longer than about 15 seconds. There is usually no effect upon the right auricle pressure. We have found that the effect upon the lung as observed by recording its volume changes is to produce a very marked congestion, the mode of production of which is in the failure of the left ventricle to empty itself against the raised aortic pressure. In consequence of this fall in output from the left side the blood in the pulmonary veins cannot flow in normal quantities into the left auricle and is therefore accumulated in the pulmonary veins and capillaries. There is no incompetence of the mitral valves. A quite analogous series of changes is also observed when other procedures for raising the systemic pressure, apart from a direct action upon the heart, have been adopted, such as stimulation of the medulla, the vagi having been previously divided, stimulation of the divided splanchnics, or stimulation of the upper surface of the spinal cord which has been divided in the lower cervical region.

We next come to the effects produced by methods which act upon the heart as well as the blood-vessels. Of these one of the most important is suprarenal extract. This is known to cause a large rise in aortic pressure due chiefly to constriction of the peripheral arterioles; but it also acts upon the heart, whose rate is increased and output nearly doubled. The effect on the heart, however, is complicated, if the vagi be undivided, by a marked inhibition of the heart occurring mainly during the period of heightened aortic pressure. The effect of suprarenal extract upon the blood-volume of the lung is to cause first a small diminution in volume, soon followed by a marked increase which persists during, and for a short time after, the rise in aortic pressure. Thus the main effect is to produce a distinct congestion of the lungs. The effect upon the pulmonary blood-pressure is a rise to nearly double its original valve, commencing with, but lasting rather longer than, the rise in aortic pressure. The left auricle pressure at first falls, but rises rapidly when the aortic pressure has reached a high point. The effect upon the right auricle pressure is a gradual and slight rise following the rise in aortic pressure. From these results we may conclude that the effects are mainly due to the action of the peripheral blood-vessels in causing the large rise in aortic pressure. They are, however, complicated by the action upon the heart. The increased output observed at first explains the preliminary diminution in volume of the lung and the rise in pulmonary pressure. The later effects are due to the rise in the aortic pressure and the consequent inability of the left ventricle to empty itself. The more reflex inhibition there is produced, the more marked, as a rule, is the pulmonary congestion. These facts have an important bearing upon the question whether suprarenal extract can be advantageously used in hæmorrhage from the lungs. Certainly in the doses which we have employed, and with intravenous injection, our experiments strongly contra-indicate its employment for such a purpose. Whether a smaller dose, given by the mouth, so that the admittance into the circulation is much more gradual, can produce a pulmonary constriction unaccompanied by a congestion is now being investigated. Some further points in regard to the action of the extract upon the pulmonary vessels are described below.

A drug of importance to us, in that it causes a fall in the systemic pressure, is amyl nitrite. This is known to produce its main effect by dilatation of the peripheral blood-vessels. The disadvantage against us here is that we are not quite certain of the details of its action upon the heart. In the cat a small dose of amyl nitrite sufficient to cause a marked fall in aortic pressure causes a large congestion of the lung, which takes place in two stages. There is a primary and moderate increase in the blood-volume, followed by a slight diminution, and this again is succeeded by the main volume increase, the nature of the record seeming to show that we are dealing with at least two different effects. The blood-pressure changes, so far as we have at present decided, have shown only slight changes, the main one being a rise in the left auricle pressure. The effect upon the pulmonary pressure is very slight and mainly in the direction of a late rise. We think, therefore, that the probable explanation of the congestion produced in the lung is a preliminary dilatation due to the increased flow to the right side of the heart, followed by a later effect due to the action upon the heart which is mainly expressed upon the left side. This fails to empty itself so completely and thus accounts for the rise in the pressure in the left auricle. In the dog the effect is not quite so striking. The changes are in the same direction but are much less in degree. There is probably a much better compensation in this animal than in the cat.

ARE THERE VASO-CONSTRICTOR NERVES TO THE PULMONARY ARTERIES ?

This question has been already worked at by many observers ; but we need here only consider the facts brought forward by Bradford and Dean and by François-Franck. All these observers came to the conclusion that there are vaso-constrictor nerves. The evidence looked for by Bradford and Dean was in one of three directions. First, a rise in the pulmonary pressure out of proportion to the aortic pressure ; secondly, a rise in the pulmonary pressure with no accompanying rise in the aortic pressure ; or, thirdly, a rise in pulmonary pressure accompanied by a fall in aortic pressure. In one series of their experiments they excited the vaso-motor centre in the medulla, having previously divided both vagi. This caused a large rise in the aortic pressure and a fairly well-marked rise in the pulmonary pressure. The spinal cord was then divided between the sixth and seventh dorsal nerves and the stimulation repeated. The aortic-pressure rise was now less than before, but there was the same rise in the pulmonary pressure. Direct evidence was also sought by stimulation of the anterior roots of the upper dorsal nerves. This caused a rise varying in amount, but never very marked, in the pulmonary pressure, accompanied at times by a fall in the aortic pressure, at other times by a rise varying with different animals and with the nerve stimulated. From experiments such as these they concluded that the only explanation of the pulmonary rise is an active constriction of the pulmonary arterioles.

François-Franck's main method was to simultaneously measure the blood-pressure in the pulmonary artery and in the left auricle, and he considers that a rise in the former, accompanied by a fall in the latter, must mean pulmonary constriction. In his experiments he stimulated the sympathetic chain in the thorax from the sixth thoracic nerve up to the ganglion stellatum. The usual results he obtained were a rise in the pulmonary pressure, a fall in the pressure of the left auricle, and, as a rule, a fall in the aortic pressure. On some occasions he observed a rise in the aortic pressure. In some of his experiments he recorded the blood-volume of the lung by a plethysmographic method, and invariably obtained an increase in volume as the result of the excitation of these nerves. This is an effect the reverse to that which we should *a priori* expect, and he supposes the result to be due to the great distensibility of the pulmonary arteries. If the arterioles are constricted over the whole lung the blood would be stored in the large vessels, and he supposes that the increase in volume thus caused is more than sufficient to compensate for the diminished volume in the capillaries and veins.

Our criticism of the results of all these experiments lies in two directions. First, the effects obtained are always very small, and all observers agree that the influence of the nerves on the blood-vessels can only be of slight degree. But the main objection to these experiments is that they have not sufficiently excluded the action of the nerves upon the heart; and until this is done it is impossible to rely on experiments of this class. Bradford and Dean consider that by taking into account those tracings only in which no apparent cardiac acceleration is to be seen any changes must be due to an action upon the pulmonary blood-vessels. Roy and Adami, however, have shown that excitation of some of the sympathetic fibres running to the heart gives rise to augmentation without acceleration, and many of our experiments have quite clearly corroborated this. If augmentation takes place without acceleration the pulmonary pressure will rise because the output from the right side is increased. The aortic pressure will also tend to rise because the output from the left side is increased; the pressure in the left auricle will fall because the left ventricle is taking more per beat; and these are the effects which we think have in reality taken place in the records of these observers. The only difficulty lies in explaining the fall in aortic pressure which they have commonly obtained. Bradford and Dean experienced exactly the same difficulty and concluded that their experiments were not sufficient to decide the cause of this fall. That augmentation has taken place in many of François-Franck's experiments is quite clear from his own tracings, for where he includes the volume-curve of the lung there is always a quite marked increase in the volume-pulse which can only indicate an increased output from the right heart.

Turning next to our own experiments we have stimulated the white rami communicantes of the upper thoracic nerves, the sympathetic chain itself, and the fibres from the ganglion stellatum. We have also attempted to exclude any possible cardiac action by a preliminary division of all the branches of the sympathetic that we could find running into the heart. We have, however, found that it is quite impossible to divide all these fibres without injuring those running to the root of the lung. We have also stimulated the fibres at the root of the lung, but in this position we have only obtained completely negative results. The usual effect of stimulating the fibres leaving the ganglion stellatum is a fall in the volume of the lung, quickly followed by an increase. In some cases we recorded an increase in the volume of the lung from the start, and in a few cases, which, however, are very rare, we have observed a diminution in the volume of the lung during the whole time of stimulation. Wherever we got a diminution in the volume of the lung we invariably found an increased volume pulse, and simultaneously a rise in the aortic pressure. We think that the whole effect is best explained by the cardiac augmentation which these results indicate, and that it is not necessary to invoke any action upon the pulmonary vessels for their explanation. In those cases in which there is an increase in volume of the lung from the start there is usually an increased volume pulse and the aortic pressure shows in many cases practically no change, and lastly in those cases, the commonest, in which a preliminary diminution is followed by an increase in the volume of the lung the increase in volume pulse is well marked, especially at first, and there is a decided rise in aortic pressure, followed by a gradual fall as the volume of the lung increases. Stimulation of the accelerator nerve itself, after it has left the ganglion stellatum, produces as a rule an increased volume of the lung, with increased volume pulse. Hence we conclude that all the results that we have obtained can also be explained in the simplest manner as of purely cardiac origin, and we therefore attempted to detect vaso-constrictor nerves, if any are present, by a different type of experiment. The nerves to be stimulated having been isolated the animal was killed by bleeding ; the blood was whipped and was placed in a reservoir. Meanwhile cannulæ were tied in the pulmonary artery and in the left auricle. One lung was then ligatured at the root. The cannula in the artery was then connected to the reservoir and blood perfused at constant pressure through the intact lung. The outflow was then recorded by a graphic method. We never obtained the least effect on stimulating any of the fibres leaving the ganglion stellatum, and hence we conclude that there are no vaso-constrictor fibres to the pulmonary vessels. Experiments of this type, we think, would certainly have given us a positive result had such fibres been present, for it is easy to obtain such results with, for instance, the nerves to the vessels of a limb.

THE ACTION OF SOME DRUGS UPON THE PULMONARY CIRCULATION.

We have already stated the general effects of suprarenal extract upon the lung and pulmonary circulation. We have also investigated the action of the suprarenal on the pulmonary arteries by direct perfusion. It gives us a very striking result ; whereas perfusion of very dilute suprarenal extract through any of the systemic vessels causes their almost complete closure, the effect upon the pulmonary vessels is in nearly all cases exactly the opposite—namely, a markedly increased rate of flow. Only in one instance have we obtained constriction, and then the effect, though marked, was in no degree comparable to that which we should obtain, for instance, upon a limb. These experiments are, of course, of importance in considering whether suprarenal extract is of value in hæmorrhage from the lungs, but our conclusions up to the present must be somewhat guarded because our experiments have not yet sufficiently decided the action of small doses. With regard to the volume changes produced in the lung by suprarenal extract the dog again shows a difference from the cat. The changes, though in the same direction, are very much smaller and the dilatation is more accentuated if the vagi have been previously subdivided—a result which we should naturally expect from the much greater and more prolonged rise of aortic pressure that occurs under these circumstances.

The action of nicotine is somewhat similar to that of suprarenal extract, though, as it exerts less effect upon the heart, its action being mainly confined to the peripheral blood-vessels, the results are rather simpler. Still, they are in the same direction—that is, to the rise in the aortic pressure there follows a congestion of the lung and simultaneously a rise in the pulmonary vein pressure and pulmonary artery pressure.

Pilocarpine gives interesting results in that it tends to produce systemic vaso-constriction, the effect of which is, however, obscured by the marked cardiac inhibition simultaneously produced. If the cardiac inhibitory fibres be paralysed by atropine, pilocarpine produces practically the same results as nicotine. When given with the cardio-inhibitory mechanism intact it produces a great increase in the volume of the lung which is due to the cardiac inhibition. As this decrease in volume takes place it must mean that the output from the left heart is less diminished than is that from the right heart. Muscarine produces the same effects as pilocarpine.

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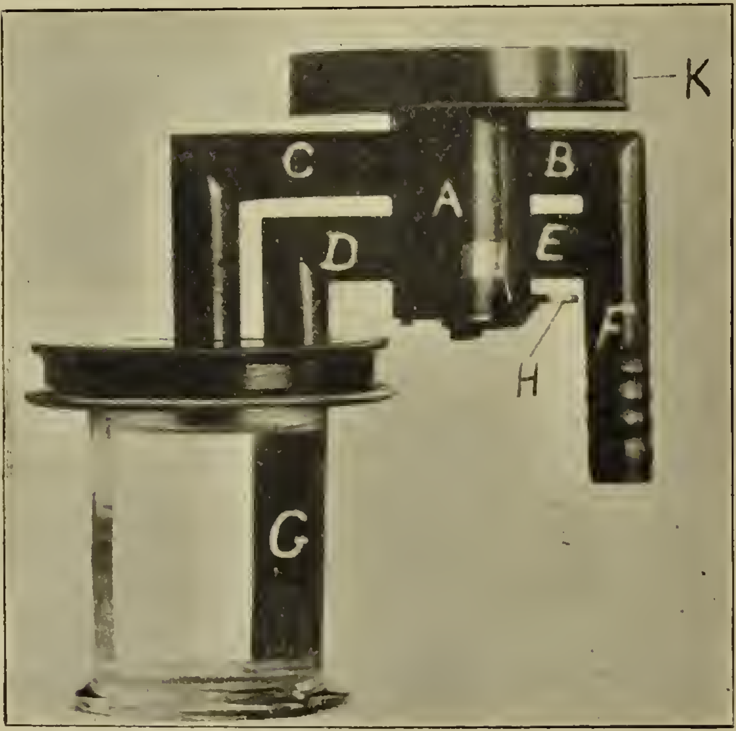
[*From the Proceedings of the Physiological Society*, January 18, 1902.]

A tap for graduating the amount of anæsthetic in experiments in which artificial respiration is being employed. By T. G. Brodie.

This consists of a cylinder *A* fitted to the end of the respiration pump and connected on the one side to a glass vessel *G* which is filled with the anæsthetic, and on the other side to a tube *F* conducting the air to the animal. The tap consists of a large outer cylinder *A* to which two tubes are fixed on either side, as in the figure, and in which a second cylinder is closely fitted. This second cylinder is divided by a horizontal septum into two chambers, an upper and a lower. The upper chamber opens directly into the respiration pump, and when the tap is pushed over to the right, a side opening in the inner cylinder corresponds to the orifice of the tube *B* of the outer cylinder. All air driven from the pump thus passes directly through *B* and *F* to the animal. As the tap is turned to the left, part of the orifice leading to *B* is closed, and simultaneously, an exactly similar amount of an orifice leading to tube *C*



is opened. Part of the air is thus diverted to the anæsthetic bottle and blows directly on to the surface of the anæsthetic. This air is conducted by the tube *D* through the lower chamber of the tap to the tube *E*, for as the tap is turned the orifices of tubes *D* and *E* are opened to the same extent as the orifice of *C* is opened. When the tap is taken quite over to the left, the orifice to *B* is closed, those to the tubes *C*, *D* and *E* are wide open and all the air passes through the anæsthetic chamber. A graduation on the outer cylinder *A*, and a pointer on the handle *H* of the tap, serve to indicate how much air is being sent through the anæsthetic bottle, and thus how much anæsthetic is being administered. The bottle unscrews from the rest of the tap so that fresh anæsthetic can be very quickly added.

The form represented in the figure screws by the collar, *K*, to the bottom of the respiration pump made, from my designs, by Mr C. F. Palmer, of Brixton.

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AN EXPERIMENTAL ENQUIRY UPON GLYCOLYSIS IN DRAWN BLOOD. By F. W. PAVY, M.D., LL.D., F.R.S., and R. L. SIAU.

(*From the Conjoint Laboratories of the Royal College of Physicians, London, and the Royal College of Surgeons, England.*)

BERNARD⁽¹⁾ in 1876 stated that sugar disappeared very rapidly from drawn blood. He asserted that dog's blood became sugar-free on standing 24 hours at room temperature.

In 1877—1879 Pavy⁽²⁾ recorded observations showing that whilst a certain amount of progressive loss occurred, it was not to be compared with that represented by Bernard, and, moreover, even after decomposition amounting to putridity had occurred, he never found that the blood reached a state to be free from the possession of cupric oxide reducing power. The cupric oxide reducing power left amounted to 0·2 to 0·3 per 1000 expressed in terms of dextrose.

The subject was revived in 1890 by Lépine⁽³⁾, who has published a considerable number of papers upon it both alone and in conjunction with Barral or Metroz, and has based upon his work a theory to account for diabetes. Lépine⁽⁴⁾ states that aseptic normal dog's blood loses on incubation at about 38° C. as much as 25 °/₀ of its sugar in one hour, whilst, on the other hand, in the blood of a diabetic person and in that of a dog rendered diabetic by removal of the pancreas, the proportion lost is very much less.

It must be noted that what Lépine⁽⁵⁾ speaks of as the "glycolytic power" of a blood is the percentage ratio of the sugar lost in one hour to that originally present, and that it is only when the loss is calculated upon this basis that diabetic blood can be said to show less glycolytic power than normal blood, the absolute loss found by Lépine being often equal in the two kinds of blood.

Harley in 1891 published⁽⁶⁾ a series of experiments showing that a variable but always considerable loss occurred. His methods of analysis, however, it may be considered must be open to grave suspicion, as the exceedingly small quantities of sugar found in some of the samples of blood employed by him are quite at variance with the experience of all other authors.

Seegen gives⁽⁷⁾ a few determinations and concludes that a loss of sugar occurs when blood is kept in a warm place, but that it is far from being as rapid as Bernard supposed.

Arthus from numerous observations concludes⁽⁸⁾ that the disappearance of sugar is a *post-mortem* effect. He finds that the loss in unit time is very slight directly after the blood is drawn, and that it slowly increases, but his values are always much lower than Lépine's. He considers that the destruction is brought about by a ferment which is generated in the blood after it has left the vessels. Colenbrander⁽⁹⁾ comes to the same conclusion.

Kraus⁽¹⁰⁾, working on other lines, Sansoni⁽¹¹⁾ and Spitzer⁽¹²⁾ conclude that Lépine's theory of diabetes based upon the glycolysis is devoid of foundation.

Experiments have recently been undertaken by us upon the matter and we will proceed to record the results obtained.

In the procedure adopted the blood was in every case drawn from the carotid of a dog anæsthetised with A.C.E. and consequently contained somewhat more than the normal proportion of sugar. About 500 c.c. of blood was collected in each case. 30 c.c. portions of blood were delivered by means of sterile pipettes into sterile conical (Phillip's) beakers provided with cotton-wool plugs, and exposed for the specified times in a room at 38° C. If sugar was added, 1, 1·5, or 2 c.c. of a titrated dextrose solution was first delivered into the beaker from a burette. In each experiment duplicate portions of 30 c.c. were put into alcohol at once and were taken to determine the original sugar content of the blood.

After the incubation was ended, 300 c.c. of methylated spirit was added to each beaker and the contents well stirred to obtain as finely divided a precipitate as possible. The sugar was then extracted and its quantity determined by the method which has now been employed by us for several years and has served as the foundation of much published work. It has been referred to in this *Journal*⁽¹³⁾ and elsewhere⁽¹⁴⁾, but as everything in relation to this question turns upon the validity of the analytical procedure adopted, it is considered advisable by us that an account of it should be here given.

After standing for at least 24 hours the precipitated proteids, &c. are stirred up in the alcohol and the beaker sunk into a water-bath and its contents thoroughly boiled. The alcohol is then poured off through a linen filter, and the coagulated proteids removed from the linen and rubbed down in a mortar to a fine powder. The powder is then returned to the beaker, 100 to 150 c.c. of spirit added, and again boiled for a few minutes, strained off, pressed, rubbed down and extracted with another 100 to 150 c.c. of spirit. The mixed extracts are placed in a distillation flask sunk in a water-bath at 60° C. and distilled under reduced pressure, nearly but not quite to dryness. To the residue in the distillation flask about 10 c.c. of alumina cream(13) and 20 c.c. of water are added, and the whole briskly boiled on a sand-bath, filtered through paper and the filtrate made up with washings of the flask and precipitate to 100 c.c. The reducing power of the clear, pale straw-coloured liquid is determined with the ammoniated copper solution in the usual way, three titrations being made as a rule.

It must be remembered that a volumetric determination is not absolute, as consecutive titrations varying by 0·1 c.c. may be obtained. When dealing with quantities of dextrose of about 50 mgrms., such a difference in the titration represents about 2 mgrms. This error in titration is to a certain extent discounted by taking a series of titrations, but nevertheless a variation of about 3 to 4 per cent. must be considered as within the limits of error of the process.

Exp. I. Dog's blood defibrinated by whipping in a covered beaker with feathers. Incubated in covered beakers. Maintained as far as possible sterile.

	Sugar in mgrms.		Difference	
	found	initial	mgrms.	per cent.
Check I	59·5	58·8	—	—
Check II	58·1			
Incubated 1 hour	55·5	58·8	– 3·3	– 5·6
,, 2 hours	53·2	58·8	– 5·6	– 9·5
,, 6¼ ,, ...	45·5	58·8	–13·3	–22·6
+ 048 g. dextrose incubated 1 hour	104·1	106·8	– 2·7	– 2·5
,, ,, ,, 2 hours	104·1	106·8	– 2·7	– 2·5
,, ,, ,, 6¼ ,,	89·3	106·8	–17·5	–16·4

Exp. Ia. The same blood but incubated in open beakers.

	Sugar in mgrms.		Difference	
	found	initial	mgrms.	per cent.
Incubated 1 hour	58·1	58·8	– 0·7	– 1·2
,, 2 hours	58·1	58·8	– 0·7	– 1·2
,, 6¼ ,, ...	42·4	58·8	–16·4	–27·9
+ 048 g. dextrose incubated 1 hour	104·1	106·8	– 2·7	– 2·5
,, ,, ,, 2 hours	100·1	106·8	– 6·8	– 6·4
,, ,, ,, 6¼ ,,	89·3	106·8	–17·5	–16·4

Here it is seen that no appreciably different result was obtained whether the blood was maintained sterile or was allowed to stand exposed to the air. It is noticeable that the loss of sugar does not in any case exceed 7 mgrms. in the first 2 hours, and that only after 6 hours does the percentage loss reach 22·6. After 6 hours' incubation also the absolute loss is found to be greater in both the portions with added sugar than in the corresponding portions incubated without added sugar, but the percentage loss (the "pouvoir glycolytique" of Lépine) is less.

Exp. II. Defibrinated sterile dog's blood.

	Sugar in mgrms.		Difference	
	found	initial	mgrms.	per cent.
Check I	47·1	47·1	—	—
Check II	lost			
Incubated 1 hour	46·3	47·1	− 1·2	− 2
,, 2 hours	46·3	47·1	− 1·2	− 2
,, 4½ ,, ...	39·1	97·1	− 8·0	−16
+·050 g. dextrose incubated 1 hour	86·2	97·1	−10·9	−11
,, ,, ,, 2 hours	83·3	97·1	−13·8	−14
,, ,, ,, 4½ ,,	78·1	97·1	−19·0	−19
+·075 g. ,, ,, 1 ,,	119·1	122·1	− 3·0	− 2·4
+·100 g. ,, ,, 1 ,,	138·8	147·1	− 8·3	− 5·5
,, ,, ,, 2 ,,	135·1	147·1	−12·0	− 8·2
+·050 g. dextrose & 1 c.c. 4 $^0/_0$ NaF ,, 1 ,,	96·1	97·1	− 1·0	− 1

Here, again, the loss in the blood incubated alone was very small in the first two hours, but it is seen to stand markedly greater in the blood incubated with 50 and 100 mgrms. of sugar. In the presence of NaF, however, no such loss is observed. After 4½ hours the loss both absolute and per cent. is greater in the blood incubated with an addition of 50 mgrms. of sugar than in the blood alone.

Exp. III. Dog's blood. Coagulation, delayed by cooling, occurred during incubation.

	Sugar in mgrms.		Difference	
	found	initial	mgrms.	per cent.
Check I	35·3	36·1	—	—
Check II	37·0			
Incubated 1 hour	33·4	36·1	− 2·7	− 7·4
,, 2 ,, ...	31·3	36·1	− 4·8	−13·3
,, 4¾ ,, ...	25·2	36·1	−10·9	−30·2
+·048 g. dextrose incubated 1 hour	87·0	84·1	+ 2·9	+ 3·4
,, ,, ,, 2 ,,	84·0	84·1	− 0·1	− 0·1
+·072 g. ,, ,, 1 ,,	105·0	108·1	− 3·1	− 2·8
+·096 g. ,, ,, 1 ,,	132·0	132·1	− 0·1	− 0·1
,, ,, ,, 2 ,,	132·0	132·1	− 0·1	− 0·1
+·048 g. dextrose & 1 c.c. 4 $^0/_0$ NaF ,, 1 ,,	87·0	84·1	+ 2·9	+ 3·4

In this case there is again a certain small progressive loss of sugar in the blood incubated alone, but in none of the portions incubated with additions of sugar is there any appreciable change to be seen.

Exp. IV. Sterile dog's blood. With 2 $^0/_0$ Na citrate to prevent coagulation.

	Sugar in mgrms.		Difference	
	found	initial	mgrms.	per cent.
Check I	36·8	37·7	—	—
Check II	38·7			
Incubated 1 hour	33·3	37·7	−4·4	−11·6
„ 2 „	37·8	37·7	+0·1	+ 0·3
+ ·048 g. dextrose incubated 1 hour	85·6	85·7	−0·1	− 0·1
„ „ „ 2 „	84·3	85·7	−1·4	− 1·6
+ ·072 g. „ „ 1 „	106·1	109·7	−3·6	− 3·3
+ ·096 g. „ „ 1 „	133·3	133·7	−0·4	− 0·3
„ „ „ 2 „	131·9	133·7	−1·8	− 1·4
+ ·048 g. dextrose & 1 c.c. 4 $^0/_0$ NaF „ 2 „	90·1	85·7	+4·4	+ 5·1

In this experiment, where citrated blood was used, a greater loss of sugar was found to have occurred in the specimen incubated for 1 hour than in any other instance in this series, but in no other portion is there a loss exceeding the limit of error of the method employed.

CONCLUSIONS.

1. The loss of sugar which occurs in dog's blood in the 1st and 2nd hours after withdrawal from the animal is so insignificant as to render it difficult to see how it can have any physiological bearing. This is opposed to what is stated by Lépine, but accords with the observations of most other writers on the subject.

2. When the time of exposure is increased to 4 or 6 hours a more definite loss is met with, but even now it does not amount to anything large. Harmony exists between these recent results and those recorded by one of us (Pavy, *Proceedings, Royal Society*) in 1879.

3. It is a part of Lépine's doctrine that the larger the amount of sugar present the larger is the loss that is found to occur (15). This receives no support from the general issue of the experiments recorded here, and as a consequence the percentage loss will be influenced by the amount of sugar that happens to be present. Upon these premisses Lépine's "pouvoir glycolytique," which represents the percentage loss, constitutes an expression which may be made to vary at will.

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(4) Lépine and Barral. Ibid. CXII. 146. 1891.

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ON THE QUESTION OF THE FORMATION OF SUGAR IN BOILED LIVER. By F. W. PAVY, M.D., LL.D., F.R.S. AND R. L. SIAU.

(*From the Conjoint Laboratories of the Royal College of Physicians, London, and the Royal College of Surgeons, England.*)

On account of statements that have been made on this point and have found their way into standard works, we have deemed it advisable to subject the matter to experiment and will here record the results obtained.

In 1876 Abeles⁽¹⁾ published the statement that sugar, in sufficient quantity to be recognisable by ordinary methods, was formed in a liver that had been previously rendered sugar-free by repeated boiling with water.

Seegen and Kratschmer⁽²⁾ state that they boiled rabbit liver with water and repeatedly washed it till the washings gave no trace of sugar reaction. They say that these last washings, after being allowed to stand 12 hours in presence of salicylic acid, gave on testing a neat reaction of sugar, and when left standing over the liver pulp, the solution became more and more saccharine. The statement is repeated by Seegen⁽³⁾ in both editions of his book on glycogenesis. Seegen⁽⁴⁾ also says that if the precipitate obtained by adding alcohol to a decoction of liver is stirred up in water, sugar is gradually produced.

Schwiening⁽⁵⁾ made a decoction of rabbit liver and incubated it for 69 hours in presence of chloroform. It was then tested for sugar and found to give a mere trace of reaction, but after having been placed aside for "many weeks" it gave with Fehling's solution a very decided reaction, much cuprous oxide being deposited. From this and similar observations he concludes that a continuous production of enzyme occurs.

In his allusion to these experiments of Schwiening, Reynolds Green says⁽⁶⁾, this conclusion "seems a little difficult to understand, as the boiling not only killed the liver cells, but presumably destroyed any antecedent of diastase as it did the enzyme itself."

In our experiments we have operated upon rabbit's and cat's livers, and as the results obtained were the same in both instances, it will suffice to give one experiment in detail.

The liver of a well-fed rabbit was excised immediately after death, minced, and thrown into boiling water. After 10 minutes the liquid was strained off through linen, and the liver squeezed, rubbed down in a mortar, and again boiled in water. This process was repeated seven times, when the extract was no longer found to give any reducing effect on Fehling's solution.

The pulp was then collected, pressed, and six equal portions weighed off into tubes which were plugged with cotton wadding and placed in the steam bath for half-an-hour to secure sterilisation. After cooling, to tubes *A* and *a* 20 c.c. of sterile water was added, to *B* and *b* 20 c.c. of 0·5 per cent. salicylic acid solution, and to *C* and *c* 20 c.c. of water and 2 c.c. of toluol.

A, *B* and *C* were allowed to stand at laboratory temperature, while *a*, *b* and *c* were placed in an incubator at 37° C. At the end of 88 hours (3½ days) the tubes were sunk for a few minutes into boiling water, and the contents after being filtered were tested with Fehling's solution. In no case, in either series, was the slightest reaction discernible.

Portions of the liver pulp were incubated with Taka diastase and also with pancreatic enzyme. On testing with Fehling's solution a reduction was obtained showing that glycogen was present.

The conclusion to be drawn from these experiments is that in a sterilised state no sugar is formed in liver substance which has been subjected to thorough boiling, and this conclusion harmonises with what our general knowledge would suggest.

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(4) Seegen. Ibid, 1900, p. 23; and Pflüger's Archiv, loc. cit.
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(6) Reynolds Green. The Soluble Ferments and Fermentation, Cambridge, 1899, p. 36.

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FATIGUE IN NON-MEDULLATED NERVES.

BY T. GREGOR BRODIE, M.D., *Director of the Laboratories of the Royal College of Surgeons* (*Eng.*) *and Royal College of Physicians* (*Lond.*), AND W. D. HALLIBURTON, M.D., F.R.S., *Professor of Physiology, King's College, London.* (Six Figures in Text.)

THE experiments, on which the assertion rests that nerve-fibres are not fatiguable, have nearly all been performed with medullated motor nerves. The method adopted in such experiments has been to excite the nerves for a number of hours, and to exclude fatigue in the terminal structures by preventing the impulses reaching the peripheral organ. On removing the block by means of which this is accomplished the activity of the peripheral organ is still manifested with undiminished force. The blocks employed have been curare[1], a galvanic current[2], the application of ether[3], and in the case of secretory fibres, atropine[4].

A few investigators have employed non-medullated nerve-fibres in their experiments. Eve[5] used the cervical sympathetic nerve in the rabbit, and after exciting this for twelve hours found that the vaso-constrictor apparatus in the ear vessels was still in action at the end of this time. The main object of his experiments was to ascertain whether any histological change is demonstrable in the cells of the superior cervical ganglion. To this end he excited the preganglionic fibres. He therefore only incidentally touched the question of fatiguability of non-medullated fibres. He was also exciting other structures (nerve-cells and medullated preganglionic fibres). But so far as can be judged from his experiments he showed that non-medullated fibres were not fatigued, and even that fatigue of the peripheral vaso-motor mechanism is difficult to demonstrate.

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[1] Bowditch. This *Journal*, VI. p. 133. 1885.

[2] Bernstein. *Pflüger's Archiv*, XV. p. 289, 1877; Wedenski, *Centr. f. d. med. Wiss.* XXII. p. 65. 1884.

[3] Maschek. *Sitzungsber. d. k. Acad. d. Wiss. Wien.* XCV. Abth. 3, p. 109. 1887.

[4] Lambert. *Compt. rend. Soc. Biol.* 10th Ser. I. p. 511. 1894.

[5] This *Journal*, XX. p. 340. 1896.

Howell, Budgett, and Leonard[1], in a paper on the influence of temperature on the irritability and conductivity of nerve-fibres, also incidentally touched upon the question; they state that vaso-constrictor and cardio-inhibitory fibres show no functional fatigue; but as the longest time during which they applied continuous excitation was one hour, their contention can hardly be considered to be satisfactorily proved.

Another set of experiments has been performed by Miss Sowton[2]. In 1897, Dr Waller[3] alluding to his well-known theory that carbonic acid is produced during the activity of nerve, writes, "You have seen that such a (medullated) nerve is inexhaustible, and yet that it exhibits very clear symptoms of chemical change after action (increase of the action current similar to that produced by minute doses of carbonic acid). All these things (*i.e.* including the facts of Wallerian degeneration) reconcile themselves with the notion that the active grey axis both lays down and uses up its own fatty sheath and that it is inexhaustible, not because there is little or no expenditure, but because there is an ample re-supply."

If this tentative suggestion is correct, and the absence of fatigue is due to the presence of the fatty sheath, fatigue ought to be demonstrable in nerve-fibres which have no fatty sheath. Miss Sowton selected the olfactory nerve of the pike as the non-medullated nerve with which to try the experiment, and the tracing she has published shows that the galvanometric replies of this nerve become slightly feebler after repeated stimulation.

As some doubt has recently been cast on the absolute trustworthiness of the electrical response as a sign of nervous activity[4], recourse to some other method appeared to us imperative.

The splenic nerve seemed to be the most convenient for this purpose, since it consists mainly of large bundles of postganglionic non-medullated fibres. Our first experiments were, therefore, conducted upon this nerve.

The following is the method of procedure we adopted:

A dog is anæsthetised with morphine and A.C.E. mixture or ether, the abdomen opened, the spleen exposed, and the splenic nerves which lie by the side of the main splenic artery are laid bare. It is quite easy to dissect out a length of nerve sufficient for the experiment (1 to $1\frac{1}{2}$ inches). The nerve is cut as far from the spleen as possible, and the spleen is enclosed in an air oncometer, as in Schäfer and Moore's[1] experiments; our instrument differed from theirs in that the splenic mesentery with its vessels, &c. enters through a long slit at the bottom of the box, instead of through a groove at the side. On stimulating the nerve with a faradic current the organ contracts and the recording lever of the writing apparatus falls. In our earlier experiments we sometimes used a Marey's tambour, or a piston-recorder. As, however, the range of these instruments proved to be too limited for our experiments we abandoned them for a light flexible bellows[2] made of cardboard and peritoneal membrane. This is brought into communication with the spleen box by a piece of stout india-rubber tubing, and can be very accurately calibrated; the amount of the fall or rise of the lever attached to the upper surface of the bellows thus gives us a measure in c.c. of the decrease or increase of the splenic volume.

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[1] This *Journal*, XVI. p. 298. 1894.

[2] *Proc. Royal Society*, LXVI. p. 379.

[3] *Lectures on Physiology. First Series. Animal Electricity*, p. 70. 1897.

[4] See Prof. Gotch's article 'Nerve' in Schäfer's *Text-book of Physiology*.

It is not absolutely necessary to use any recording apparatus at all; the shrinkage of the spleen when the nerve is excited is perfectly evident to the eye, and is limited to that portion of the spleen which

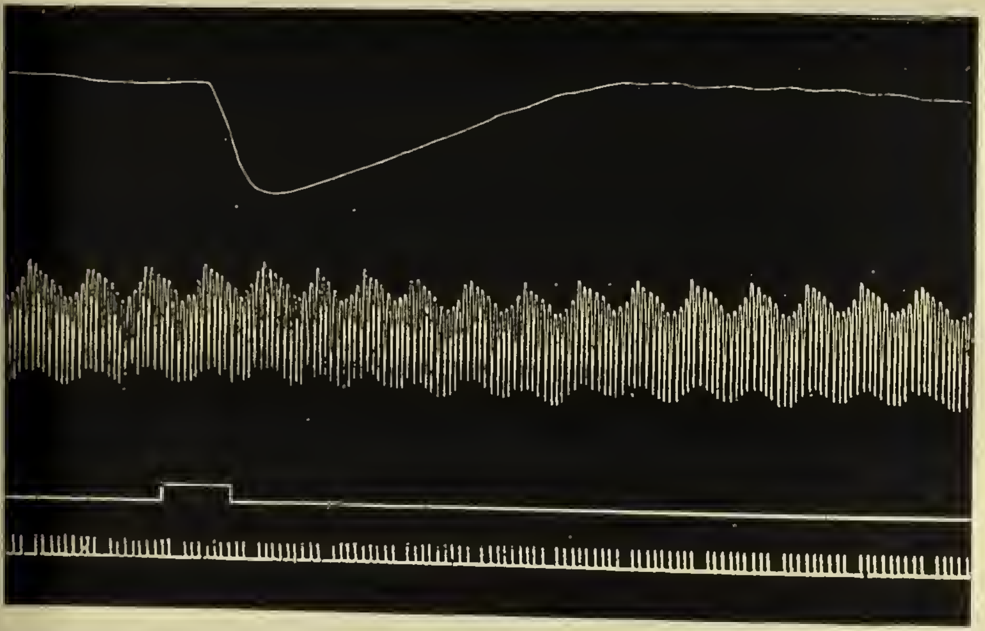

Fig. 1. Dog. Morphine and ether. The upper line gives the splenic volume, the second the carotid blood-pressure. The signal line is 6 cms. above the zero of blood-pressure. Time tracing in seconds. During the time indicated by the signal a large splenic nerve was stimulated. Coil at 2 cms. The decrease in volume of the spleen amounted to 6 c.c. This tracing is the same size as the original.

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[1] This *Journal*, xx. p. 1. 1896.

[2] This piece of apparatus was exhibited by T. G. Brodie at the International Congress of Physiologists. Turin, 1901. See also this *Journal*, xxvii. p. 473. 1902.

is innervated by the nerve-fibres on the electrodes. Still it appeared to us advisable to employ the exact method of registration just described, as it gave us quantitative differences in the contractions recorded at different times.

Simultaneously with the splenic tracing we always took a tracing of the arterial blood-pressure from the carotid artery. This we did because most of our experiments lasted many hours, and the condition of the arterial pressure furnished us with a convenient means of gauging the general condition of the animal. We give (Fig. 1) a typical tracing of the result obtained on stimulating the nerve.

A slight rise of arterial pressure usually occurs simultaneously with the contraction of the spleen (see Fig. 3, *D*), an effect the cause of which is discussed later (p. 197).

We next found that a longer stimulation does not keep the spleen contracted, but that the organ gradually returns to its original volume in spite of the stimulation. The production of this condition is also clearly shown by recording the successive contractions produced by short excitations repeated after brief intervals of rest. This is shown in the next tracing (Fig. 2).

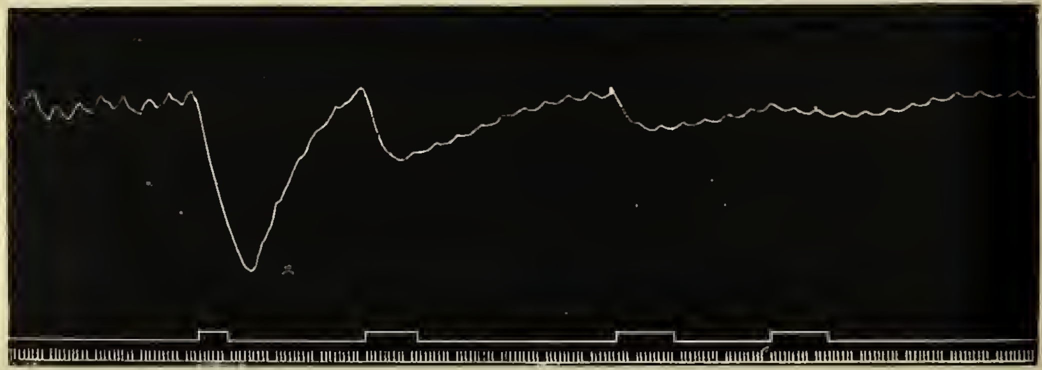

Fig. 2. Dog. Morphine and ether. Splenic volume. At the times indicated by a rise of the signal line a splenic nerve was stimulated. The strength of the stimulus was the same in all four instances. Du Bois coil, 2 Leclanché batteries, coil at 6 cms. Time tracing records seconds. This figure is rather more than half the size of the original tracing. The first contraction caused a diminution in volume amounting to 14 c.c.

Here it will be seen that the effect of each stimulation is smaller than the preceding, and in the end hardly any response is noticeable. This is due to some change analogous to fatigue in the terminal structures, for the fourth excitation of the series in the record (Fig. 2) was applied to a piece of nerve nearer to the spleen than that which was excited during the first three excitations. That it was not due to fatigue of the nerve-trunk was excluded by our subsequent experiments.

It was, therefore, evident that we must have recourse to some form of block so as to prevent the nerve-impulses reaching the spleen. Curare and atropine are both ineffective, and so we tried the constant current. This was sent in by means of platinum electrodes, placed between the exciting electrodes and the spleen, and it proved a very effective block. But it has this disadvantage; the polarisation of the nerve lasts so long that a considerable time elapses after the removal of the current before the fibres can once more conduct nerve-impulses. After quite a weak current ($\frac{1}{3}$ of a Daniell cell) has been sent in for two minutes the nerve does not transmit impulses for an hour or more; it then slowly recovers. If, therefore, faradic excitation of the nerve is kept up all the time the constant current is in, and fails to cause a contraction of the spleen when the constant current is removed, it is impossible to say whether this is due to fatigue of the nerve-fibres on the proximal side of the block, or whether it may not be due to the fact that the block created by the constant current is still effective.

Non-medullated fibres are much more sensitive to the polarisation effects of a constant current than medullated nerves are. We made control experiments on the dog's sciatic nerve; the block created in the sciatic passes off much more rapidly, and even when six Daniell cells are employed has disappeared in twenty minutes after five minutes' exposure. The prolonged injurious effect produced by a constant current was observed by Wedenski, who avoided it by reversing the direction of the current at fairly frequent intervals. We tried this with our non-medullated nerves, but were never able to abolish the effect, so as to be certain the block was removed immediately the current was broken.

We had, therefore, to employ a block which would be at once effective and immediately removable. We found that cold fulfils these requirements admirably. The nerve rests on a pair of platinum electrodes, and on a metal tube through which cold brine can be kept flowing. This tube is interposed between the electrodes and the spleen. The electrodes and tube are mounted on a small block of wood which can be easily kept in position, and the nerve is kept moist by a cover of thin rubber. If the temperature of the fluid is just about freezing point[1], nerve-impulses are completely blocked. Immediately the cold fluid is replaced by warm (36° C.) the nerve conducts the nerve-impulses again.

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[1] As the nerve and tube lie within the body the fluid is warmed several degrees when circulating through the tube; it is therefore necessary to employ a colder solution (a few degrees below zero) than would suffice to block the impulses if the nerve was freely exposed to the air.

We ascertained first the effect of these variations of temperature without using the exciting current. The effect is almost inappreciable; sometimes we have noticed a slight slow expansion of the spleen when the cold saline is turned on, and sometimes an increase in the amplitude of the splenic waves. Turning on the warm solution subsequently produces the opposite effect, but this is always extremely small.

If the brine in the tube is kept at the low temperature mentioned, and the nerve is being excited with strong induction shocks all the time, the spleen remains irresponsive; the nerve-impulses are able to reach the block but not to pass it. If now warm water is passed through the tube, thus removing the block, and the spleen continues to be irresponsive, we have a proof that the piece of nerve between the electrodes and the tube has been fatigued.

Our experiments have, however, shown us that the splenic nerves are just as difficult to fatigue as medullated motor fibres. We have done a number of experiments in which we varied the length of time during which the nerve was stimulated, but even after the longest period (six hours) the nerve is practically as excitable as it was at the start, for a good splenic contraction is obtained when the cold block is removed. One of our numerous graphic records is shown in the next tracing (Fig. 3).

At the commencement of this experiment the spleen gave the contraction reproduced in Fig. 1. The duration of the stimulus was 9 seconds and the secondary coil was 2 cms. from the primary. The nerve was next blocked by cold, and after a short time the secondary coil was pushed home on the primary, and the stimulation continued with short intervals at the end of each hour to test whether the nerve remained excitable. After the total duration of the excitation had amounted to 3 hours the tracing of Fig. 3 was taken. At the beginning of this tracing the nerve was still being stimulated. At *A* the current of cold saline was stopped and replaced by warm water at *B*. It is seen that 7 seconds later the spleen commenced to contract. The stimulation was stopped at *C*, and 44 seconds later, *i.e.* at *D*, the nerve was again excited for 10 seconds when a very marked contraction resulted. This contraction was larger than that of Fig. 1, for as already pointed out the strength of the stimulus had been increased a little. In this experiment the nerve was still further excited, until the sum of the times of stimulation amounted to 4½ hours but still with the same result.

From experiments such as this, and from others in which the time of excitation amounted to more than 6 hours, we conclude that the splenic nerve-fibres cannot be fatigued.

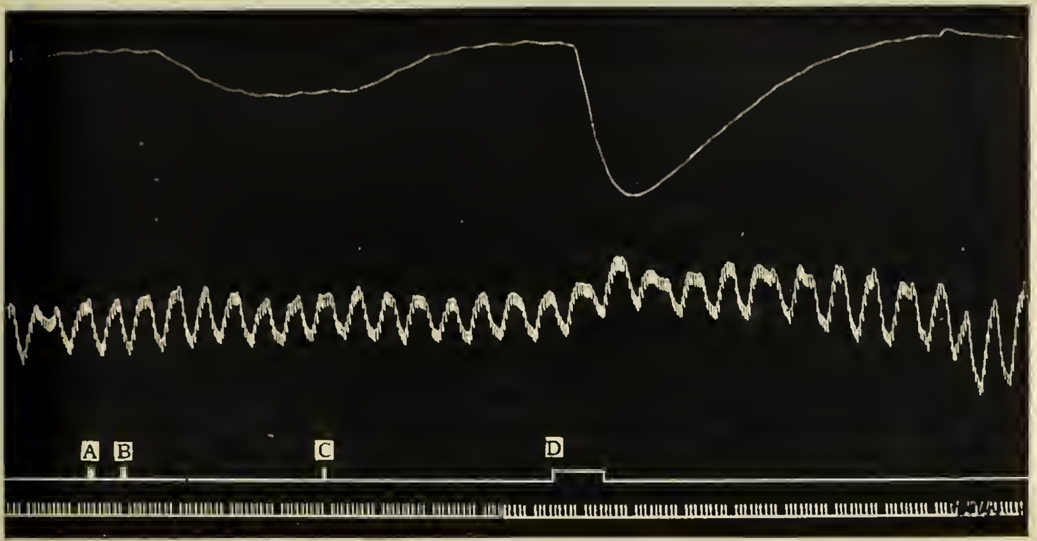

Fig. 3. Dog. Morphine and ether. A large splenic nerve has been excited continuously for three hours. This excitation is still continued at the beginning of the tracing. The upper tracing is that of the splenic volume. The second, the blood-pressure taken from the carotid. The next line is a signal line. The zero-abscissa of blood-pressure is 5 cms. below this line. The time record marks seconds. This figure has been reduced to about two-thirds of the original size.

We examined histologically some of the nerves which had been excited, and found the vast majority of the fibres were non-medullated, but that there were always a few fine medullated fibres. In one specimen, for instance, we found as many as 50 medullated fibres, whereas the non-medullated were present in thousands. In reply to a letter from us regarding these fibres Dr Langley kindly wrote to us as follows:

"The medullated fibres peripheral to the solar plexus are partly afferent, partly preganglionic, which have not yet reached their cell-stations (very few of these go to the spleen), and partly medullated post-ganglionic. They all, but especially the last, vary in number in different animals."

"The external carotid branch of the superior cervical ganglion in the rabbit, which amongst other structures supplies the ear, contains very few medullated fibres, half-a-dozen perhaps, and possibly these are all afferent."

Even if all those fibres of the splenic nerve which are medullated were motor it is hardly possible to imagine that the effect we obtained could be due to them, having regard to their insignificant number, and the very large area of the spleen which contracts.

A possible source of fallacy in our experiments was pointed out by Prof. Gotch at a meeting of the Physiological Society when we gave a demonstration on this subject. He suggested that the distance between the exciting electrodes and the cooling tube, *i.e.* five millimetres, was possibly too short, and that the effect of the cold might spread to the position of the exciting electrodes, and annul the excitability of the nerve to faradic stimulation at this spot. If this were so we should not have been starting nerve-impulses at all, and our method would, therefore, be useless for determining the problem. Since then we have performed another experiment, which has convinced us that Professor Gotch's objection will not hold. The experiment to test the point is very simple, and consists in reversing the position of the cooling tube and the electrodes. The cold brine was kept flowing for 46 minutes, and its temperature 0° C. at first was depressed until it reached – 5° C. (the outflow water having a temperature of – 3° C.)[1]. The effect did not spread to the exciting electrodes; and at each test excitation during this period, between one and two hours, the spleen executed as large contractions as it had shown before the cold brine was allowed to run.

We have extended our observations to other nerves, and our first experiments in this direction were performed upon the cervical sympathetic.

The external carotid branch was dissected out, and left in connection with the superior cervical ganglion; all the other branches of the ganglion were divided. The nerve in question breaks up into twigs around the artery very soon, so that it is not possible to isolate more than about half-an-inch: the ganglion forms a convenient handle by means of which the nerve can be lifted on to the electrodes. We at first placed the nerve beyond the electrodes on a tube through which cold brine was kept flowing, and found that cold here as in the splenic nerves formed a convenient and perfect block to the impulses. If no block is employed some amount of constriction of the ear vessels persists throughout the period of excitation, and flushing of the ear takes place when the stimulation ceases. In one experiment we continued stimulating for six hours, with occasional brief stoppages to observe the relaxation.

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[1] These are temperatures considerably lower than those we have found it necessary to employ in our experiments.

We found that some constriction persisted during the whole period of excitation, but that the constriction gradually decreased, and the longer the stimulation was kept up the less pronounced became the final flushing.

We therefore thought it better to test the question in a region where it would be possible to obtain a graphic record of the result. Accordingly we selected the hind leg of the dog as the subject of our experiment. The animal was anæsthetised in the usual way, and sufficient curare was given to prevent the contraction of voluntary muscles on stimulation of their nerves. The limb was enclosed in a plethysmograph, and the sciatic nerve was exposed and stimulated. The resulting fall of the plethysmograph lever persists for about a minute, but in spite of the continuance of the excitation the lever gradually ascends and in a period varying from five to ten minutes has nearly returned to its original level. When the faradisation is stopped the plethysmograph lever rises above its previous level, showing that vascular dilatation has occurred. If a few minutes are now allowed to elapse, a second period of excitation leads practically to the same result. If only half-a-minute's interval is allowed, the constriction is less marked, and the return is more rapid. But there is usually a small amount of residual constriction, and dilatation occurs on cessation of the stimulation. The excitation was kept up in one animal for 4½ hours, with occasional brief intervals to see if dilatation occurred. In another animal the excitation was maintained for 5 hours with a similar result.

The results we had obtained upon the blood vessels of the rabbit's ear and the dog's leg led us to still further extend our experiments to other vessels and we therefore performed some upon the intestine. The splanchnic was exposed at the lower part of the thorax by resection of the posterior portions of two ribs. About 1½ to 2 inches of the nerve were then dissected out and having been cut centrally the nerve was placed upon shielded electrodes. A loop of intestine was enclosed in a plethysmograph and its volume changes recorded by a small bellows-recorder. The results of these experiments are in the main similar to those we had found with the sciatic. The constriction is at first great and remains so for a variable time. In our experiments this time has varied from 3 to 8 minutes. A gradual dilatation then sets in and

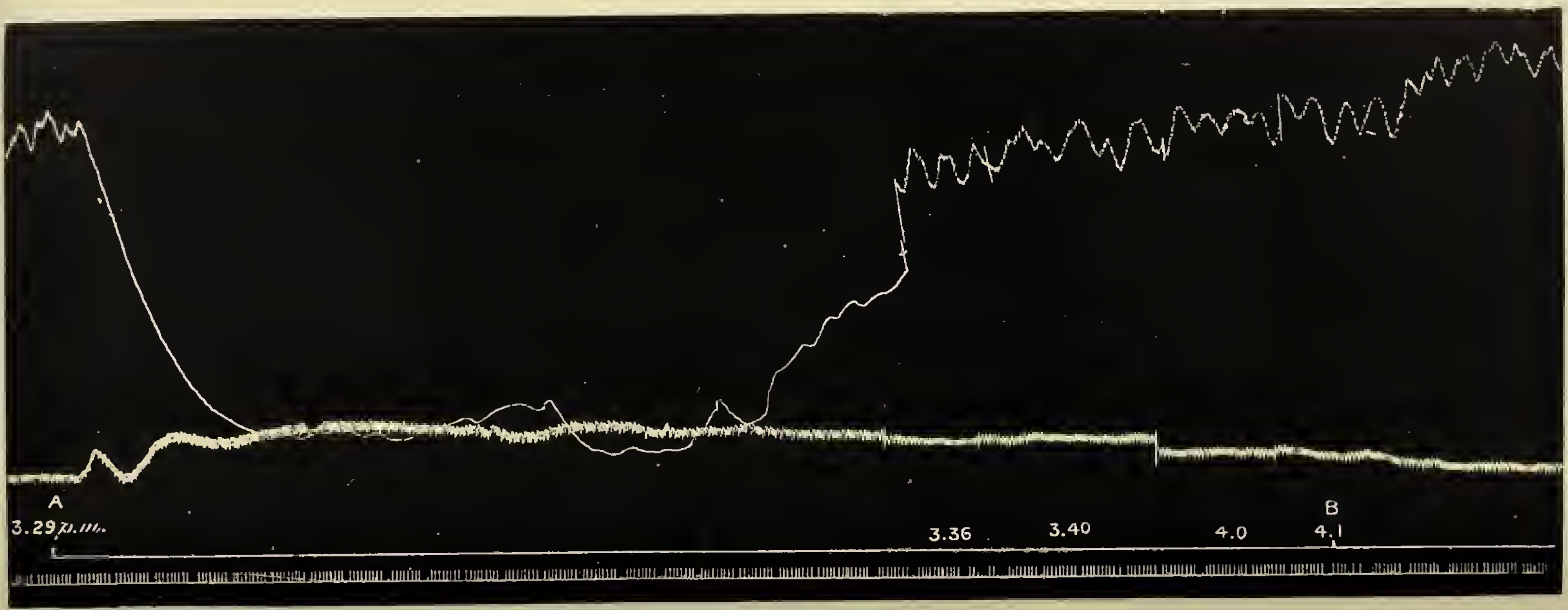

Fig. 4. Dog. Morphine and A.C.E. mixture. The uppermost line is a tracing of the intestinal plethysmograph; the next is blood-pressure. Time tracing shows seconds. At *A* on the signal line faradic excitation of the splanchnic nerve was commenced (3.29 p.m.). The intestinal vessels almost immediately contracted and the lever of the bellows-recorder fell, and remained in this position for about 3 minutes; the two large movements noticed during the last minute of this time were produced by slight struggling movements of the animal. The lever then began to gradually ascend, and short pieces of the record were taken at intervals; the time is shown under the tracing; at 4.1, *i.e.* 32 minutes after the observation began, the stimulation was stopped (*B* on signal line), and a slight further relaxation of the vessels took place. This figure is rather more than half the size of the original.

after a time varying from 6 to 25 minutes the organ has nearly returned to its original volume. If the stimulation is stopped soon after this stage is reached a further dilatation is recorded which may lead to a rise of the recording lever to a higher level than that at the start of the experiment. If the stimulation be continued for a considerably longer time this dilatation is much less marked and may even be absent. The result of a typical experiment is shown in Fig. 4. If the nerve is again stimulated within a minute of the cessation of the first stimulation, as a rule no contraction is recorded, and if any occurs it is very small in amount. If, however, we wait about 15 mins. and then stimulate again, we record just the same series of changes as before but with the following differences. The amount of contraction is only about one-half that of the first, is slower in onset, and recovery begins much earlier. Even in these cases, however, it is common to observe a small amount of dilatation when the stimulation is stopped. In a number of experiments of this kind we have repeated the stimulations several times until the total duration of excitation has added up to between 5 and 6 hours, and we have still been able to observe, in a few cases, a dilatation on cessation of the stimulation, but the longer the time of stimulation has been the less marked is the ultimate dilatation. This is well seen in Fig. 5. We have in all cases controlled our results by inspection of the intestine itself. The blanching with the first excitation is of course very evident and so is the dilatation which comes on during the course of the stimulation, but the dilatation occurring when the excitation is stopped is at best very difficult to observe, and in many instances was quite undetectable although a reference to the record proved that one had taken place.

We see, therefore, that in all our experiments upon blood vessels we invariably found that the constriction passed off in spite of a continuance of the excitation, and that this was especially the case when the splanchnic or the sciatic was stimulated. Also, on cessation of stimulation there may be no further dilatation (Fig. 5), though usually some was recorded. When obtained it was never great in amount, rarely exceeding $\frac{1}{10}$th of the amount of the original constriction. Since the completion of our experiments we have found that the same effect has been observed by Howell, Budgett, and Leonard[1] for several different sets of sympathetic fibres. They, too, record it for the blood vessels of the hind limb when the sciatic is stimulated, and have

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[1] *Loc. cit.*

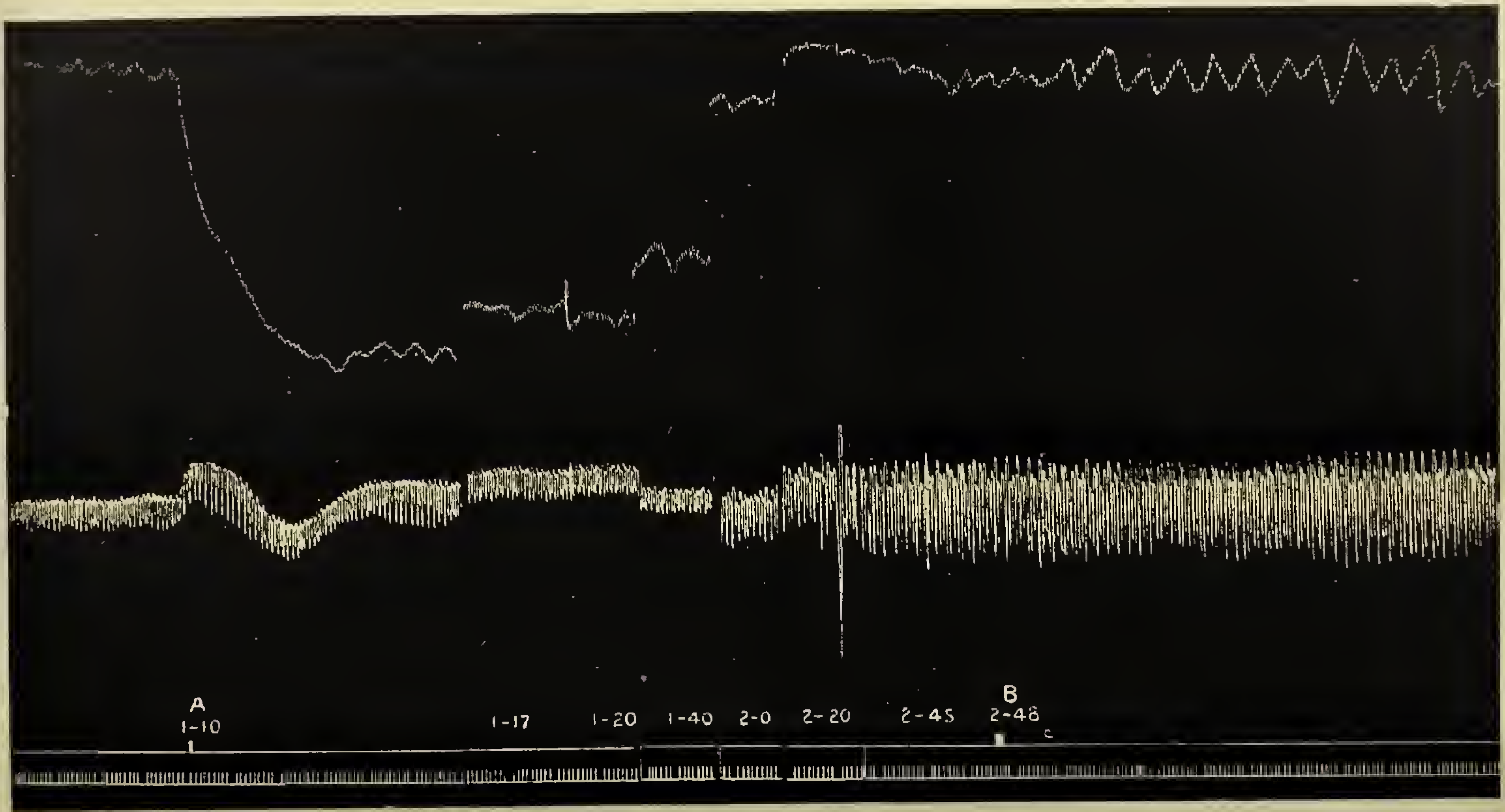

Fig. 5. Dog. Morphine and A.C.E. mixture. The uppermost line is a tracing of the intestinal plethysmograph. The next is blood-pressure. Time in seconds. At *A*, 1.10 p.m. the right splanchnic was excited and this excitation maintained until *B* at 2.48. The tracing gives short lengths of tracings at successive intervals of 20 mins. It is seen that dilatation had commenced at 1.17, and was complete at 2.20. The figure also shows that there is no dilatation occurring after cessation of the stimulus at *B*. This figure is two-thirds the size of the original.

observed a similar effect when watching the dilatation of the pupil following stimulation of the cervical sympathetic in the rabbit.

In attempting to determine to what cause this effect is due we have several possibilities to consider. It may be a peripheral effect, *i.e.* it may be due to fatigue of the muscle or nerve endings. Or again, it may be a local effect upon the nerve at the spot excited, or an effect upon the whole length of nerve down to the vessels. If a local effect upon the nerve it may be due to mechanical injury, to polarisation, or to local fatigue. Of these possible causes we consider that a local polarisation due to the current is the most probable explanation, though there are indications that other factors take some part in its production. Thus we found that, if the electrodes are shifted nearer the periphery after we had excited the nerve for a time long enough to allow dilatation to occur, a good constriction could be obtained. This constriction was only in rare cases as extensive as the original, but was usually less in amount and slower in development, indicating as we think some degree of fatigue either of the muscles or nerve endings. If, after a prolonged stimulation, we tested the different parts of the nerve we found, as above stated, that stimulation below caused constriction, stimulation at the original spot caused no effect even if the strength of stimulation was increased, and finally that stimulation above the original level also caused constriction in the majority of cases. In this latter case the constriction was never quite so marked as that produced by exciting below.

The condition of the nerve therefore was, that it was inexcitable at the original spot stimulated, but excitable above and below that spot, and further that though inexcitable it could still conduct, but to a somewhat less degree, than in the parts which had not been stimulated. Howell noted that the effect was mainly a local one and pointed out that by moving the electrodes peripherally a further good contraction could be obtained. They speak of the effect as "stimulation fatigue," but the use of the term fatigue in this connection is we think scarcely a wise one. We have so frequently observed the extreme sensitiveness of these non-medullated fibres to electrical currents that we are much rather of the opinion that we are here dealing with a polarisation effect. This is also supported by the fact that the nerve just below the part stimulated is still excitable and gives no evidence of fatigue. To avoid polarisation we performed experiments in which we used the Helmholtz modification and non-polarisable electrodes, but here again obtained the same results as before. We also excluded the possibility that they were due to the use of too strong a current of stimulation or to mechanical pressure on the nerve.

In order to avoid any possibility of polarisation we have attempted mechanical and chemical stimulation but have not been successful. All the forms of mechanical stimulation have proved most ineffectual. Thus stimulation by dropping mercury on the nerve or by v. Uexkull's tetanometer very rarely excited at all, and if any effect was produced it was minimal. Pinching the nerve is effective, but is obviously useless for our experiments for it destroys the nerve and the stimulus lasts but a short time.

With regard to chemical stimulants, we obviously could not employ any reagent which would affect the nerve injuriously. Of the chemical agents employed by previous workers, sodium fluoride appeared to us to be the one we were in search of, since its effects can be abolished by washing it away with physiological salt solution. We used solutions of this salt varying in strength from 1 to 4 per cent. but with absolutely negative results. Dilute sulphuric acid also caused no excitation. It may be mentioned here that one of us (T. G. B.) has tried dilute acids and alkalis, sodium sulphate, sodium chloride, and magnesium sulphate of various strengths upon the vagus in the cat but in all cases with negative results.

It is a very curious thing that different nerves vary in the readiness with which 'stimulation fatigue' may be produced. Of the nerves tested by Howell, Budgett, and Leonard it was shown most clearly by the vaso-constrictor and sweat fibres of the sciatic nerve. Vaso-constrictor fibres at their origin from the cord, and in the cervical sympathetic where they are medullated do not exhibit this phenomenon. Such a distinction between medullated and non-medullated nerve-fibres was, however, not found to be of universal application; for instance 'stimulation fatigue' was as readily produced in the sweat fibres in the part of their course where they are medullated, as in the part where they are non-medullated.

To this we have to add, that various non-medullated fibres show a similar difference; the non-medullated splenic nerves do not show any 'stimulation fatigue' even after six hours' excitation, or if any is at all noticeable it is exceedingly slight. In the investigation of this phenomenon there is of course no necessity to have recourse to a block. After the completion of our experiments on the sciatic and splanchnic nerves we returned once more to the splenic nerves, and some experiments without a block fully confirmed our earlier results; little or no stimulation fatigue was discoverable. In the case of the splenic nerves 'peripheral fatigue' is much more easily produced.

When we consider how insensitive mammalian nerve-fibres are to chemical, mechanical, or thermal stimuli, whereas they respond very readily to electrical stimuli, it becomes probable that electrical stimulation must produce some very considerable disturbance at the spot excited. If this is so, we can readily understand that prolonged stimulation by electrical currents of high potential may produce marked injury at the excited spot, especially if the nerve is a very sensitive one. In this connection, too, it is important to emphasize the point already noted, that though a nerve in which "stimulation fatigue" has been produced is inexcitable it can still conduct. That is, that though it cannot respond to the very abnormal mode of excitation by an electrical stimulus it can still carry on its normal and proper function, viz. that of conduction, in which the spot is excited by the proper physiological impulse.

To sum up, although we are inclined to regard the phenomenon of 'stimulation fatigue' as probably due to the injurious polarisation effects produced by electric currents at the spot of nerve stimulated, we are unable to be quite positive that this is the correct explanation, first because of our inability to control our experiments by the methods of mechanical or chemical stimulation, and secondly because we are unable to explain the difference just noted between various nerves.

The main object of our research was to test Dr Waller's hypothesis that the presence of a medullary sheath explains why fatigue in nerve-fibres cannot be demonstrated. By the use of our methods of investigation we have shown that functional fatigue is just as difficult to produce in non-medullated as in medullated nerves.

Although some recent observers[1] have once more affirmed that the non-medullated fibres possess a thin medullary sheath, we consider that Tuckett[2] has satisfactorily proved that this is not the case.

We are therefore convinced that Waller's hypothesis cannot hold as a correct generalisation for all nerve-fibres.

On *a priori* grounds we should not have expected to find that non-medullated fibres are peculiarly susceptible of fatigue, seeing that some of them, especially the vaso-constrictors, are continually in action, and that dilatation of the vessels always occurs when they are cut.

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[1] Gad and Heymans. *Arch. f.* (*Anat. u.*) *Physiol.* p. 531, 1890; Ambronn and Held. *Arch. f. Anat.* (*u. Physiol.*) p. 201. 1896.

[2] This *Journal*, xix. p. 267. 1895–6.

Certain side issues of our work have attracted our attention; one of these is the remarkable sensitiveness of non-medullated fibres to the polarising effect of a constant current; another is the rise of arterial blood-pressure which accompanies splenic contraction; and a third is the question whether on prolonged activity non-medullated fibres become acid.

We will take these three points one by one.

1. The duration and intensity of the blockage created by quite weak constant currents has already been alluded to. Two minutes' exposure to a current equal to one-third of a Daniell cell creates a block which lasts for more than an hour, and yet in the case of the same nerve (the splenic nerve with which most of our experiments have been performed, and in which so-called 'stimulation fatigue' is not a noticeable phenomenon), several hours' excitation with alternating induction shocks produced in the usual way by the Wagner's hammer of the inductorium has very slight polarising effects. We thought it worth while to ascertain whether a succession of induction shocks all in the same direction would produce an effect comparable to that caused by the constant current. In order to do this we used the key previously described by one of us[1]. In some experiments we used only make shocks, the break shocks being all short-circuited. In others we used break shocks only. We found, as we anticipated, that the effect produced is comparable to that caused by the constant current, though it naturally takes longer to develop. We used the same induction coil as before with two cells, the secondary coil being pushed home. The rate of stimulation was about twenty times per second.

In one dog, the piece of splenic nerve excited was polarised, or to put it another way, manifested loss of excitability due to 'stimulation fatigue' after 35 minutes' excitation; in another animal about an hour and a half was necessary to produce the same effect. The block created in this way extends some little distance (three or four sixteenths of an inch) beyond the excited spot and lasts for about an hour. We consider that such experiments support the hypothesis that 'stimulation fatigue' is a polarisation phenomenon.

The sensitiveness of non-medullated nerve to electrical currents suggests to us two possible explanations of the effect observed by Miss Sowton on the non-medullated fibres of the pike's olfactory nerve. In the first place the diminution she found in the galvanometric response in this nerve after repeated stimulation is probably not an indication of true functional fatigue. For by repeated closure of the galvanometer circuit the nerve is being repeatedly submitted to the influence of a weak galvanic current, namely, the action-current of the nerve itself, and the diminution of the amount of galvanometric response may be simply the result of the polarising effect of a constant current. In the second place, she may have been dealing with an instance of 'stimulation fatigue.' She at any rate does not mention in her paper that she shifted the position of her exciting electrodes.

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[1] T. G. Brodie. *Proc. Physiol. Soc.* p. x. 1896. This *Journal*, XIX.

2. The second side issue of our work to which we have devoted attention is the rise of blood-pressure which occurs on stimulating the peripheral end of the cut splenic nerves.

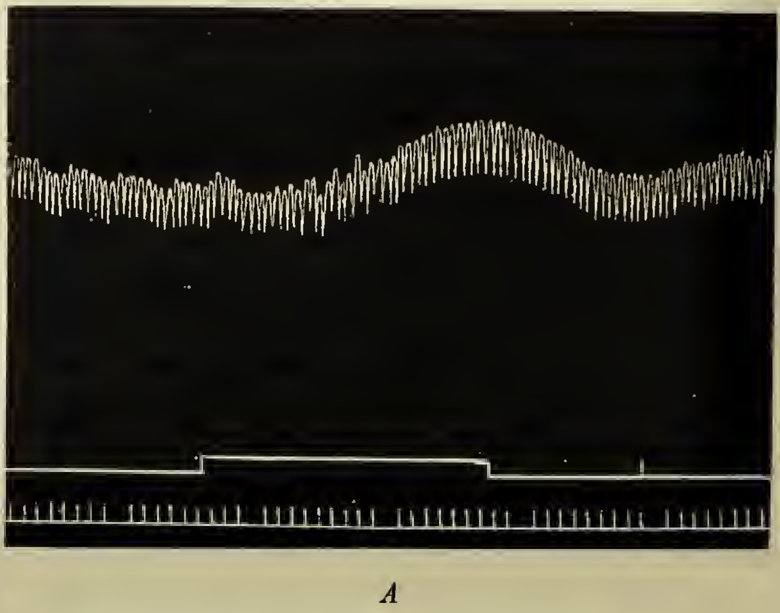
Using the calibrated bellows recorder, the amount of blood squeezed out by the contracting spleen into the general circulation was found to average 10 to 15 c.c. We doubted at first whether this slightly increased flow, acting through the liver and pulmonary circuit, could be sufficient to account for the rise of carotid blood-pressure observed, but we found on actually testing the point that this simple explanation is the correct one, and that it is unnecessary to suppose that there is any nervous reflex concerned. We injected 10 c.c. of defibrinated blood into the splenic vein at a rate comparable to that produced by the contracting spleen, and invariably found a slight rise of arterial pressure similar to that seen in our previous tracings.

Fig. 6 shows the graphic record of such an experiment; in *A*, the rise of arterial pressure which accompanied a splenic contraction (equal to 10 c.c.) is shown; *B* shows the similar effect produced in the same animal by injecting 10 c.c. of defibrinated blood into the splenic vein.

Physiological salt solution injected in the same way produces a similar result. In one dog we employed, this rise of arterial pressure was always in the case of salt solution, even if warmed to body temperature, followed by a marked fall; the same occurred if the injection was made into the crural vein. We have very seldom noticed this effect in other animals, and we are unable to account for it; no doubt this particular animal was in some way peculiarly sensitive to salt solution. Defibrinated dog's blood produced no such fall.

3. We tested the reaction of the stimulated nerves, thinking that here was a favourable opportunity of ascertaining whether on long-continued excitation there is a development of acid similar to that described by Eve and others in the central grey matter. The absence

of acidity in medullated nerve might be possibly due to the masking effect of a mass of myelin. We, however, never found that the splenic nerves even after the longest stimulation became acid to litmus. We did not investigate the question of carbonic acid production. There was also no histological difference between the portion of the nerve which had been excited and that which had not.



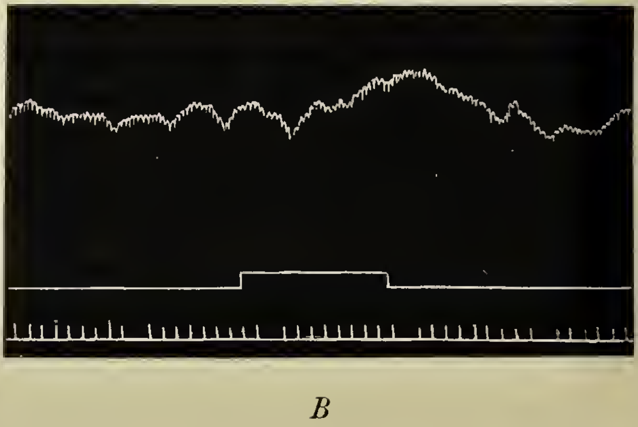


Fig. 6. Dog. Morphine and ether. Tracings of carotid blood-pressure; time in seconds. *A*. During the time indicated by the rise of the signal line, excitation of a splenic nerve caused contraction of the spleen; in *B*, during the time indicated by the rise of the signal line, 10 c.c. of defibrinated dog's blood was injected into the splenic vein. In both *A* and *B* there is a slight rise of pressure. Original size.

GENERAL CONCLUSIONS.

The main results of our work are the following.

1. Using cold as a block in the same way that Bowditch used curare and Bernstein a galvanic current in their well-known experiments which showed the non-fatiguability of motor medullated nerve-fibres, the non-medullated nerves of the spleen even after many hours of stimulation give no demonstrable evidence of fatigue.

2. Non-medullated nerve-fibres do not become acid to litmus as the result of the prolonged stimulation described in our experiments.

3. The small rise of arterial blood-pressure which occurs soon after the splenic contraction begins is due to the increased flow through the liver produced by the increased output of blood from the contracting spleen.

4. In experiments on fatigue in non-medullated nerves, the constant current is not suitable as a blocking agent. Non-medullated fibres like those in the splenic nerve are remarkably sensitive to the constant current and do not transmit nerve-impulses for a considerable time after quite weak currents have been passed through them for short periods. Induction shocks in one direction produce a corresponding polarising effect only more slowly, but if one uses induction shocks in alternating directions any appreciable amount of 'stimulation fatigue' does not occur in the splenic nerve even after several hours' excitation.

5. The term 'stimulation fatigue' introduced by Howell, Budgett, and Leonard indicates that certain nerves (mainly but not entirely of the non-medullated variety) are injuriously affected by prolonged faradic stimulation, so that the spot which has been excited is no longer excitable until a considerable period of rest has elapsed.

6. We have noticed in the spleen that 'peripheral fatigue' is induced somewhat readily. We have seen but little evidence of this in vaso-motor nerves; the vaso-motor nerve-fibres we have mainly tested are those in the sciatic and splanchnic nerves. These nerves do not manifest signs of 'functional fatigue' in the course of the nerve-fibres. They, however, differ from the splenic nerves in that the phenomenon of 'stimulation fatigue' is produced with comparative readiness.

7. A portion of nerve in which 'stimulation fatigue' has led to a complete loss of excitability can still transmit nerve-impulses, though its power in this direction is somewhat impaired.

8. We think that the use of the term fatigue in this connection is not correct. We are inclined to believe that it is due to the injurious polarisation produced by electric currents. We were not however able to establish this hypothesis by control experiments in which mechanical or chemical excitation was employed, for non-medullated fibres show little or no response to this form of excitation, unless the mechanical means adopted are such as to injure the nerves. We are also unable to explain why different nerves like the splenic and the intestinal should manifest so great a difference in the readiness with which this so-called 'stimulation fatigue' is manifested.

9. The diminution of the action-current observed by Miss Sowton after repeated stimulation of the non-medullated nerve-fibres of the pike's olfactory nerve is probably not an indication of true functional fatigue, but is possibly produced either by the polarising effect of a weak constant current due to the closure of the action-current through the galvanometer circuit, or else is a case of 'stimulation fatigue.' Possibly a combination of both factors comes into play.

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ON RECORDING VARIATIONS IN VOLUME BY AIR-TRANSMISSION. A NEW FORM OF VOLUME-RECORDER.

By T. G. BRODIE, M.D., *Director of the Laboratories of the Royal Colleges of Physicians and Surgeons, London.* (Eleven Figures in Text.)

(*From the Laboratories of the Royal College of Physicians, London, and the Royal College of Surgeons, England.*)

THE recording of variations in volume of any part of the body has been attained in many ways, which we may conveniently consider as falling into two groups, in the first of which the organ is surrounded by a liquid, and in the second by air. In the first of these groups fall the earlier observations, such as those of Fick, Mosso, and Roy. To Schäfer and Moore is due the credit of first employing air in the transmission of volume-changes, and since then it has been almost universally adopted. The disadvantages in the employment of liquid for transmitting volume-changes are chiefly two. The first lies in the difficulty of ensuring that the pressure in the liquid at the surface of the organ remains constant throughout the whole experiment, and in keeping this pressure low. It is impossible to avoid the first of these two objections when rapid movements have to be recorded. The second great disadvantage entailed in using liquid is due to its great inertia, and it is chiefly from this cause that the older methods of recording have been discarded.

In employing air-transmission for recording variations in volume either a tambour or a piston-recorder has been used as the receiving instrument. But sufficient attention does not seem to have been paid by workers to the great difference entailed by the use of these two instruments. Many experiments with which I was engaged led me to attempt to make an instrument possessing a far greater range than the ordinary forms of piston-recorder obtainable, and the testing of the instrument I finally devised led me to work out many points of detail which are briefly described in the following paper.

To record volume-changes accurately it is clear that the moving parts of the recording instrument must possess as little inertia as possible and must follow volume-changes without setting up changes in pressure. Variations in volume can, of course, also be estimated by recording the variations in pressure set up by the compression or rarefaction of the enclosed air, but in order to deduct with any accuracy the real volume-changes, it is necessary, in each experiment, to calibrate the recording apparatus by writing the variations in pressure set up by injecting known volumes of air into the air-space. A tambour can only be employed in this manner, for the range within which it may be regarded as a volume-recorder is extremely limited. This range varies with each tambour, and depends upon the diameter of the tambour, the thickness and tension of the rubber covering it, and finally with the total volume of air contained within the tambour, connecting tubing, and receiving tambour or oncometer. A tambour is in fact a pressure-recorder rather than a volume-recorder. This is well seen from the following experiment.

A small bottle, of about 150 c.c. capacity, was connected by rubber tubing 5 mm. in internal diameter to a water manometer and to the tambour. This tambour was 30 mm. in diameter and was covered by a rubber membrane 0·2 mm. thick. Successive quantities of water, each 0·5 c.c., were injected into the bottle, and after each injection the pressure in the manometer was read. The successive readings were 8, 16, 32, 50 and 64 mm. of water. Thus the pressure rises quickly to a considerable height.

The same tambour was next covered with very thin rubber (0·04 mm. thick) and the experiment repeated. The pressures then obtained were 5, 10, 14, 18, 22 and 27 mm. of water. These changes of pressure are so considerable that for oncometric experiments in which a moderate range of movement is to be recorded it is very difficult, even impossible, to make the oncometer air-tight. Moreover these relatively high pressures must exert an effect upon the venous outflow.

For these reasons and because it can never give a direct record of volume-changes a tambour should not be used in most oncometer work.

For obtaining a direct record of volume-changes we have, therefore, up to the present, possessed only one instrument, viz. the piston-recorder. The first to make use of the general principle of this recorder was Roy[1] in his various forms of oncograph. A simple form was devised by

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[1] Roy. This *Journal*, III. p. 206. 1880.

Schäfer[1] for recording the contractions of the isolated frog's ventricle. In both these instruments a liquid, oil, was used for transmitting the volume-changes, and therefore they possessed the disadvantages already described. The first to invent a piston-recorder registering by air-transmission was Ellis[2], and a little later a modification of this was employed by Johansson and Tigerstedt[3]. The instrument was carefully tested by Hürthle[4], to whom is due the form now commonly employed. The original instrument of Ellis is extremely sensitive, that is, it will respond to very minute volume-variations, but his pattern requires very careful handling. Recently, a more durable form has been described by Lombard and Pillsbury[5], who also have used it for recording minute changes. For experiments of this class there is no other instrument which can be employed.

For very many purposes the piston-recorder works admirably, but it possesses certain disadvantages. The first of these is the junction between the piston and cylinder. In order that the piston may move very freely it must not fit the cylinder too closely. To render it air-tight it is therefore necessary to seal this junction with oil. In a long experiment this requires a good deal of attention, and moreover oil is continually leaking into the lower part of the cylinder, thus introducing an error into the volume-record. Very commonly too oil finds its way into the rubber tubing leading to the oncometer. The oil also damps the instrument, thus leading to the obliteration of many of the finer movements in the record. Recently Hürthle has devised a form in which oil may be dispensed with. The piston is a light vulcanite cup, as in the old pattern, but it works in a metal cylinder which it accurately fits. This is stated to be quite air-tight for all ordinary purposes and one is directed to use no oil. The instrument which I have received is by no means air-tight and is quite valueless for long experiments in which an accurate volume-record is required. One is therefore obliged to use oil and then the friction is considerable, because the fit of the piston in the cylinder is too close. Unless the recorder is quite air-tight the piston tends to move in the direction in which the movement being recorded takes place more quickly.

But the greatest disadvantage of the piston-recorder is its limited range. Thus in the latest Hürthle recorder, which is provided with three sizes of piston and cylinder, the greatest available range for the smallest cylinder is 4·5 c.c. For the medium-sized cylinder it is 8 c.c., and for the largest 14 c.c. For such purposes as, for instance, recording volume-changes of the spleen during stimulation of its nerves, in which a diminution of volume amounting to 15 c.c. is often obtained, or in recording the output per beat of the heart these pistons are useless.

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[1] Schäfer. *This Journal*, v. p. 130. 1884.

[2] Ellis. *Ibid.* vii. p. 309. 1886.

[3] Johansson and Tigerstedt. *Skan. Arch. f. Physiol.* i. p. 345. 1889.

[4] Hürthle. *Pflüger's Arch.* liii. p. 301. 1893.

[5] Lombard and Pillsbury. *Amer. Journ. Physiol.* iii. p. 186. 1899.

The want of an instrument which would record considerable volume-changes and yet remain sensitive led me in the first instance to attempt the manufacture of piston-recorders of large diameter. They proved most unsatisfactory, and after attempting several modifications I had to abandon them. One form which gave moderately good results consisted of a very light cylinder, made of thin celluloid, whose upper end was closed and the lower end sealed by dipping into a water cup. The instrument however possessed too much inertia, and thus the level of the water varied considerably, the water moving rather than the cylinder. To avoid this I used, instead of water, mercury covered with a layer of saturated mercuric chloride solution, the latter in order to reduce the surface tension of the mercury. This gave much better results, but was not altogether satisfactory.

THE BELLOWS RECORDER.

After many further attempts I finally hit upon the idea of making the receiver of the recorder in the shape of a small pair of bellows. This form has proved in many respects of great advantage. The only difficulties experienced in devising them were, in finding a material sufficiently strong, light and flexible, and in making them quite air-tight. These have been overcome by employing peritoneal membrane for the parts where flexibility is required, and by varnishing them with a dilute solution of boiled linseed-oil, which only slightly affects the flexibility.

Fig. 1 gives a picture of one of these recorders.

They are made in the following way. A base of metal, wood, or vulcanite, through the centre of which an inlet tube opens is first made. The base is rectangular, the ratio of the long to the short side being 3 : 2. A rectangle of the same size is then cut from a thin sheet of aluminium, and the centre of this is cut away, leaving only a rim of about 5 to 10 millimetres width. This is then covered with paper which is fixed to it by dilute spirit varnish. This forms the top of the bellows and it is then hinged to the base along one of the shorter sides. For this hinge I have found it best to employ a very thin flexible leather. This is fixed to base and cover by Prout's glue, leaving about 5 mm. of leather between the two[1]. The leather is fixed so that it will lie entirely within the bellows when finished. A piece of peritoneal membrane free from holes is next chosen and cut roughly into the required shape. I have usually made the sides of such a size that, when the bellows are completely distended, each side is an equilateral triangle, and the face opposite the hinge is thus of the same size as the cover. A piece of thin cardboard of the size of the cover is taken and the peritoneal membrane is folded round this so as to form creases, which enables one more easily to fix it to the cover and base in the proper position. To fix the membrane I have found it best to use dilute spirit varnish. The membrane is first fixed at the end, the sides are then folded over, creased, cut if necessary to the right width and varnished down. If the amount of membrane is not sufficient to cover the whole of the hinge an extra piece is fixed in that position. In using the spirit varnish it is very important that those parts of the

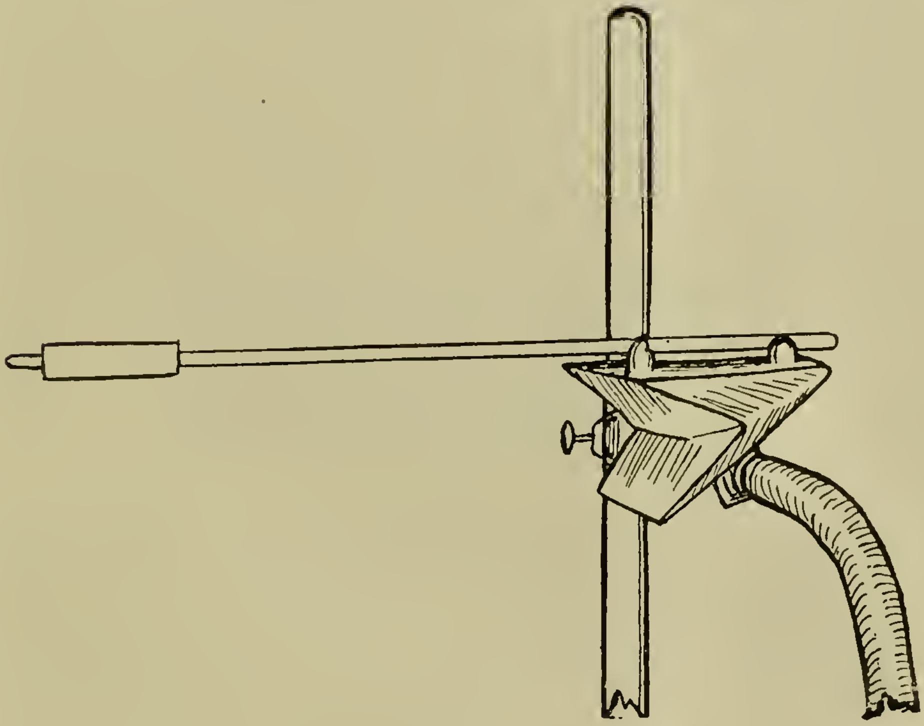

Fig. 1.

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[1] In fixing the leather to the base and cover no cement in the form of a solution can be used, for this soaks into the leather, which thus becomes stiff on drying.

membrane which are to form joints in the bellows should be untouched by the varnish so that they may remain quite flexible. This is especially important at the hinge. When the spirit varnish has thoroughly dried the bellows are folded. The folding should be carried out so that the face opposite the hinge folds inwards to lie between cover and base, whereas the sides are folded outwards (see Fig. 1).

The whole is now coated with a varnish made by diluting boiled linseed oil with three times its volume of xylol. Two or three coats of this are applied at intervals of about half-an-hour. This makes the whole air-tight, but the flexibility still remains. Should the bellows at any time become too stiff all that is necessary is to paint them with xylol, and if they leak a coat of the drying oil varnish will, if they are properly made, at once render them air-tight again. They may be used while still wet if care be taken that the wet surfaces do not touch one another.

In making large bellows-recorders I find it of advantage to make those parts which do not bend of paper, rather than of peritoneal membrane, because the latter is rather too pliable in large surfaces. All the bending parts must be made of the more flexible peritoneal membrane.

I have given so many of the details of the manufacture of the recorders because it is quite easy to make them for oneself. They are also now being made by the Cambridge Scientific Instrument Company.

Air blown into the bellows lifts the cover which rotates about the hinge, and to record these movements is very simple. No further joint is required—all that is necessary is to attach a writing lever of the necessary length to the cover of the bellows at right angles to the hinge. If it is wished, the cover and writing lever may be counterpoised by a small weight, but this only adds to the inertia, and I therefore usually work without one.

The advantages of this form of recorder are the following:—

(*a*) It is always ready for immediate use and requires no preliminary adjustment.

(*b*) It wears well and is not easily damaged.

(*c*) It is extremely sensitive.

(*d*) It can be made of any capacity, and even with very large ones the weight of the moving parts is minimal.

The last point is, perhaps, its greatest advantage. I have at present in use four different sizes. The smallest is 30 mm. long and 20 mm.

wide and possesses a capacity of 7·5 c.c.; the second is 45 by 30 mm. with a capacity of 25 c.c.; the third is 60 by 40 mm. with a capacity of 67 c.c.; and the largest is 120 × 80 mm. and possesses a capacity of 500 c.c.

The smallest of these recorders is suitable for recording the volume-changes of such organs as the kidney, intestine, or the limb of a small animal, and shows the details of the tracing well. This size is approximately of the same sensitiveness as the smallest piston of the Hürthle recorder. In Fig. 2 is reproduced a tracing yielded by a short length of dog's intestine.

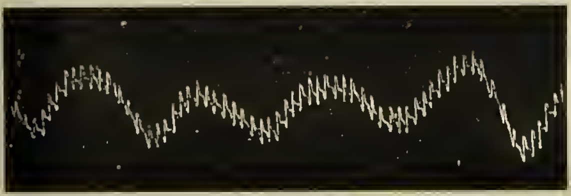

Fig. 2. Volumetric tracing of a loop of dog's intestine. The large waves are due to pendulum movements of the intestine. The small waves are due to the heart-beats. Taken by smallest bellows with magnification 8-fold.

The second size of recorder I have used for cardiometric tracings in cats or rabbits. Fig. 3 gives such a tracing, which illustrates the effect of suprarenal extract upon the output of the cat's heart. It is seen

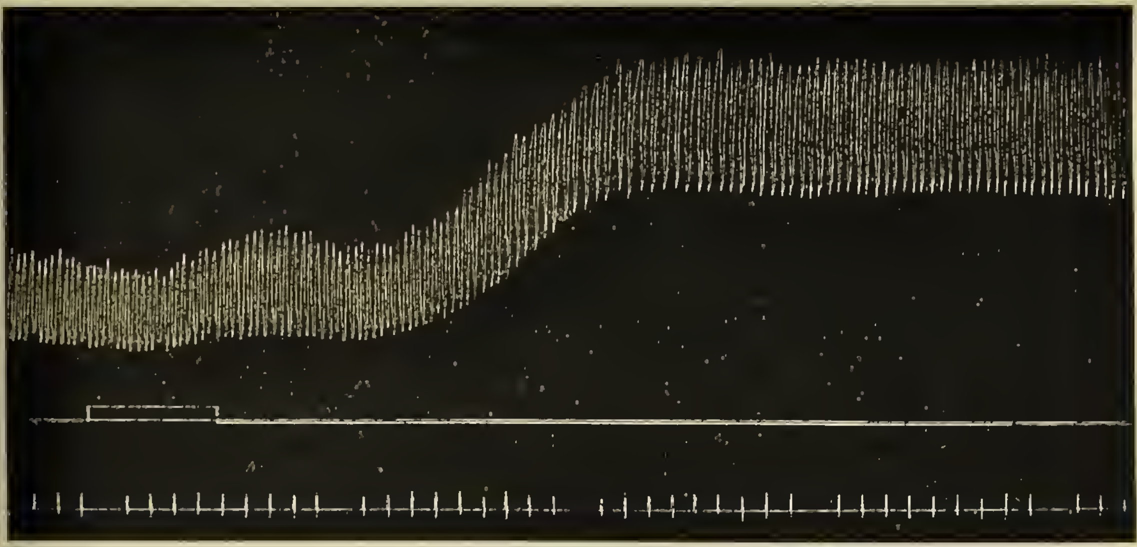

Fig. 3. Cat, anæsthetised with ether. Cardiometric tracing. A downward movement means decrease in volume. At the time indicated by the signal a watery extract from 2 gr. of dried suprarenal was injected. Time record in seconds. Second size of bellows. Magnification 5-fold.

that the output per beat is increased, that the heart becomes considerably distended and at the height of that distension is markedly slower in rate.

I have used the third size of bellows for recording the contractions of the spleen in medium-sized dogs, or for recording the volume-changes of a lobe of the lung. Fig. 4 gives a tracing of the contraction of the spleen of a dog on excitation of one of its nerves. The total contraction amounted to 14 c.c. In this figure the magnification is very small, and therefore the tracing only shows the splenic waves without any indica-

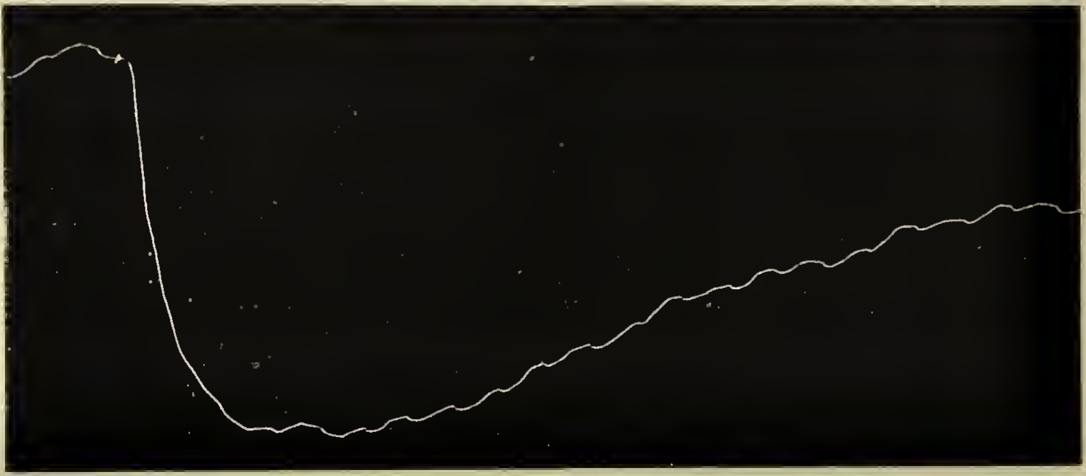

Fig. 4. Medium-sized dog. Ether and morphia. Splenic volume. Contraction on exciting splenic nerve. Total volume-decrease 14 c.c. Third size of bellows Magnification 3-fold.

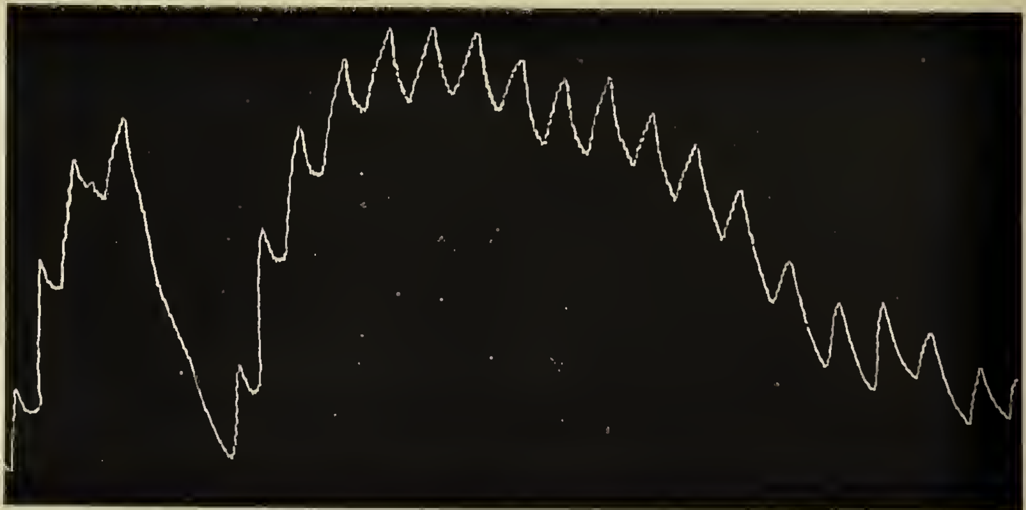

Fig. 5. Same experiment as in Fig. 4, but with smallest bellows. Magnification 6-fold. The total amplitude of the large splenic wave is 2 c.c.

tion of the heart-beats or respiratory waves. For comparison I therefore give Fig. 5, taken from the same animal immediately after the tracing of Fig. 4, but using the smallest bellows. In this the volume-changes due to heart-beat and respiration are very evident.

The largest bellows, I have employed in recording the total air movements in respiration in small animals. Fig. 6 is such a record from an experiment upon a cat. The animal was anæsthetised and a cannula tied in the trachea. This was connected by a short piece of wide rubber-tubing to a large bottle of 7 litres capacity. From this again a second tube led to the bellows. Inspiration is represented in the tracing by a down-stroke. The extent of the tracing indicates that the air inspired at each respiration amounted to 90 c.c. An interesting feature of the tracing is that it shows variations of amplitude occurring in a regular series. So far as I am aware these waves have never been observed in a respiratory tracing. They are universally present in records obtained by this method and are now being investigated.

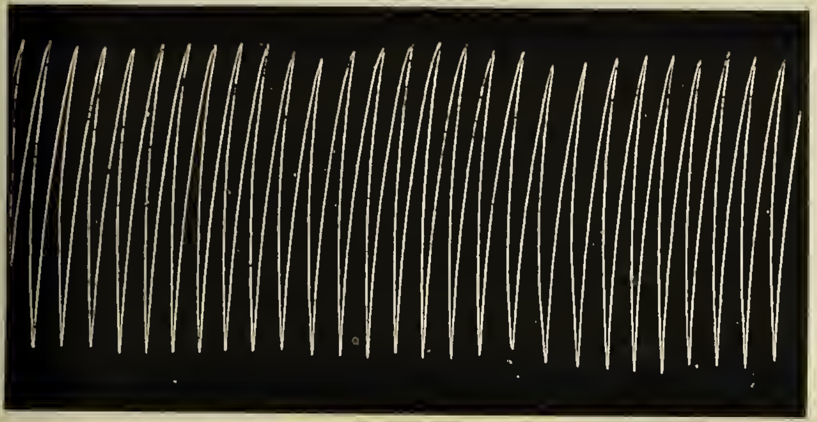

Fig. 6. Total air-volume movements in respiration of a cat. Inspiration is recorded by a downward movement. Amplitude of movement 90 c.c. Largest bellows. Magnification 2-fold.

The only form of apparatus which in any respect resembles the bellows-recorder is one invented by Gad[1] for recording the inflow and outflow of air in respiration. This instrument, which Gad terms the "Aeroplethysmograph," consists of a hollow wedge made of mica which rotates about one edge as a hinge. The lower edges of the wedge are immersed in water, thus enclosing an air-space into which the animal is made to breathe, a large air-space being interposed between the animal and the recorder.

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[1] Gad. *Arch. f. (Anat. u.) Physiol.* p. 181. 1879.

Having found that the recorder gave what were apparently very accurate tracings in many experiments, I next carried out a series of tests with the object both of comparing it with the piston-recorder and of studying the general problem of recording volume-variations by air-transmission. At the outset there were several points involving the bellows only, which had to be examined. The first of these is; is it necessary to counterpoise the cover and writing-lever? It will, of course, be best to avoid the use of a counterpoise if possible and thus reduce the inertia of the moving parts.

The only possible error in working without a counterpoise is that there will always be a positive pressure within the bellows and oncometer during an experiment. This is however of no importance if that pressure be small, and remain constant. I therefore measured it for the four bellows described, and found it to be as follows:—In No 1 (*i.e.* the smallest) it lies between 2 and 3 mm. of water; in No. 2 it is just less than 2 mm.; in No. 3 it is 1 mm.; and in No. 4, 0·5 mm. of water. The pressure is the same for all positions of the cover. In all cases the same writing-lever was employed. It is 240 mm. long and weighs 0·12 gr. It consists of a thin strip of cane 2 mm. wide and 0·22 mm. thick. To its end is gummed a strip of thin writing-paper 20 mm. long and 5 mm. wide. To the end of this is fixed a small bent glass writing-point. The use of the writing-paper gives greater flexibility and enables the writing-point to follow accurately any unevenness of the recording surface. By this writing-lever the magnification with the smallest bellows is 8-fold, *i.e.* the movement of the writing-point is 8 times greater than that of the free edge of the cover of the bellows. This edge, of course, moves more than any other point of the cover.

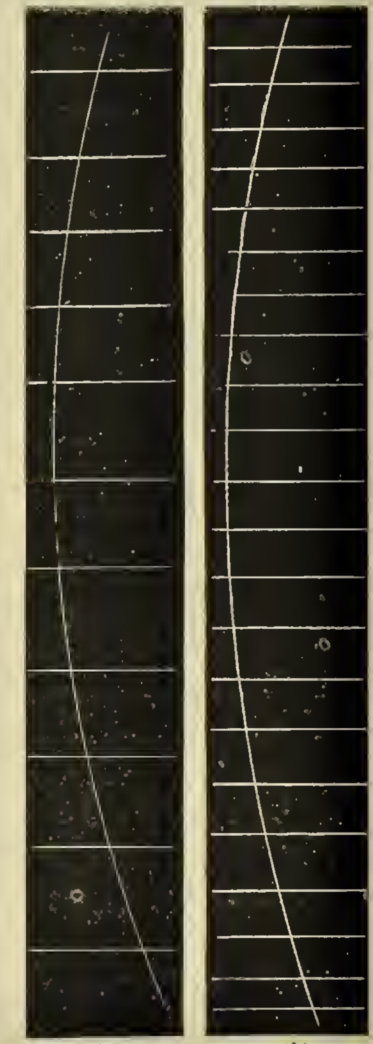
(*a*) (*b*)

Fig. 7. (Reduced by ¼.) Calibration of the first and third sizes of bellows. In (*a*) the volume-increase between two horizontals is 0·5 c.c. (smallest bellows); and in (*b*) it is 2 c.c. (third size of bellows). In both cases the lever was 24 cms. long.

The pressures caused by omitting to counterpoise are in all cases very small, and as they do not vary in different positions, we may conclude that the recorders can quite safely be employed without counterpoising.

The next point tested was whether the recorder gave equal excursions in all positions for equal variations in volume. I found that if the bellows be made so that the sides are in the ratio of 3 : 2, and if the sides be folded as described above, the lever gave very nearly equal increments of movement for equal increments of air injected. This was tested by injecting equal volumes of water into a bottle, the air displaced being carried to the recorder. Examples of the tracings obtained in this manner are given in Fig. 7. In this tracing (*a*) was given by the smallest bellows, the successive horizontal lines indicating differences of 0·5 c.c. of volume. Tracing (*b*) was given by the third size of bellows, and gives differences of 2 c.c. between two successive horizontals. It is seen that the excursions are fairly equal throughout the whole range. A similar tracing yielded by a piston-recorder gives the same result.

In tracing (*a*), the magnification is 8-fold ; in tracing (*b*), 4-fold.

EXPERIMENTS FOR TESTING ACCURACY AND SENSITIVENESS.

In the next series of experiments tests were carried out to determine whether the recorder always gave the same excursion on injecting a fixed volume of air, both for different positions of the bellows and on different days. Many such measurements have been made, and they show that the recorder may be completely relied upon in this direction.

The next test was to find how quickly the recorder would respond to known volume-changes. The rapidity at which the recorder can work accurately is largely dependent upon the inertia of its moving parts; but it is also necessary that the connecting tubing should not offer too much resistance to the air-movements. A simple and most accurate test of the manner in which a recorder works consists in observing the air-pressure variations in the recorder, tubing or oncometer. A theoretically perfect recorder should show no measurable variations in pressure. I have tested the accuracy of the recorder in the following manner. A small pump was made to drive a known volume of water in and out of an enclosed space connected to the recorder and to a water-manometer. The pump was driven at varying rates and the movements of the recorder written upon a moving surface. The oscillations of the manometer were read at the same time.

Experimenting in this way it was soon found that irrespective of the recorder two other factors play a most important part. The first is the internal resistance of the air in its movements in the tubing, and the second is the nature of the writing-point. In the case of movements running a slow course, the resistance to the air is of little moment and we may therefore use a long length of tubing of even small diameter (2 to 3 mm.). With quick movements it is essential to employ wide tubing, and further, in order to diminish as far as possible the error due to the fact that the variation travels as a wave along the tube, it is necessary to keep the tube as short as possible. Thus in actual experiments I have found that in taking a cardiometric tracing the tubing leading to the recorder should certainly be not less than 10 mm. in diameter. In respiration experiments it should be 20 or even 30 mm. in diameter.

The second condition is to make sure that the writing point is quite rigid in the vertical direction. Thus the writing-point above described (p. 482), while excellent for small variations and for slow changes, is not suitable for rapid movements of considerable extent. The use of a thin strip of paper does not give it sufficient rigidity in the vertical direction. I have found it best to replace it by a fine writing-point of thin quill such as is used in Hürthle's recorders, or in Verdin's patterns of the Marey tambour.

Having satisfied these conditions we may now return to the experiment the arrangement of which was described above. In the final tests of this kind the tubing leading from the pump to the recorder was 42 cms. long and 10 mm. in internal diameter. In describing the results, for the sake of comparison, similar experiments are given for the piston-recorder and tambour.

(1) With the smallest pattern bellows-recorder the variations in pressure in the manometer, when volume-changes of 1·5 c.c. amplitude were rhythmically produced 80 times per minute, were 1 mm. of water. When the rate was increased to 160 per min. the oscillations were 0·25 mm. (In this case the rate of change was too quick for the manometer to accurately follow the pressure changes.)

(2) With the smallest piston of the Hürthle recorder the variations were 1·5 mm. and 0·5 mm. respectively.

(3) With a tambour 30 mm. in diameter and covered with rubber membrane 0·22 mm. thick the variations were 14 mm. and 4·5 mm. respectively.

In this experiment therefore there is a slight advantage in favour of the bellows over the piston-recorder, and both are far better than a sensitive tambour.

The next two figures (8 and 9) give the result of the test with regard to accuracy in recording the volume-change. The magnification of the record in both cases is five-fold. In tracing (*a*) of the two figures the rate at which the pump was worked was 80 per minute, in (*b*) it was 125 per minute, and in (*c*) 160 per minute. It is seen from the two figures that, with the first two rates, both bellows and piston give accurately all the details of the rhythmic change and there is no alteration in the amplitude of the excursion. With the faster rate both recorders fail to give the complete amplitude, as judged from the first one or two excursions which were recorded while the motor driving the pump was working up to its full speed. There is seen to be very little difference between the behaviour of the two recorders under the conditions of this experiment.

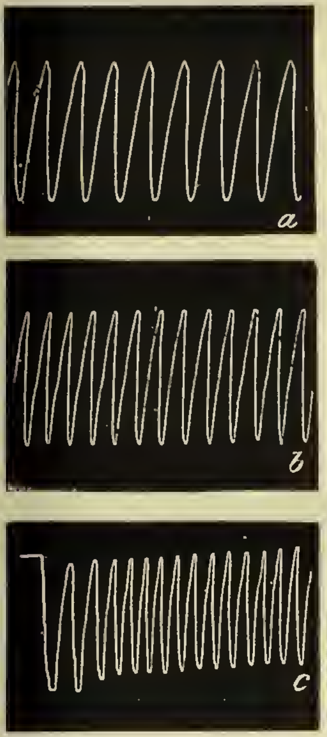

Fig. 8.

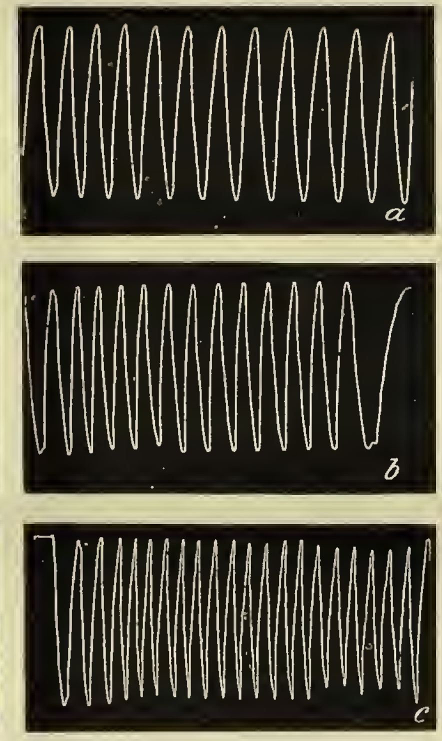

Fig. 9.

If the two are tested with volume-changes of less extent both follow the movement completely with much faster rates of pumping. Thus with movements 0·5 c.c. in amplitude both gave the full records even when the rate of pumping was increased to 215 per minute. When the rate was increased still further both finally failed, the piston-recorder a little earlier than the bellows-recorder.

A further modification of this mode of testing, which I have employed, consisted in observing the variations in pressure caused by very suddenly driving air into the recorder. This was effected by allowing a weight to suddenly fall upon and compress a wide rubber-tube connected to the manometer and recorder. The volume of the air driven in, in one of these experiments, was 3·2 c.c., and caused an oscillation in the water-manometer of 4 mm. water with the smallest bellows, and of 9·5 mm. with the small piston-recorder.

In addition to testing the accuracy and rapidity with which the two recorders will respond to fairly large volume-changes I have also tested them with respect to sensitiveness, *i.e.* as to how small a change they will accurately record, and whether they will still follow minute changes when the time occupied by the variation is progressively diminished. Perhaps the most searching test of this character is that employed by Marey[1] in testing tambours. This consists in transmitting the vibrations of a tuning-fork to the recorder to be tested. The tuning-fork sets in movement the membrane of a transmitting tambour and these oscillations are transmitted by air to the tambour or recorder. With especially light levers and very sensitive tambours Marey succeeded in recording 250 vibrations per second by this method. With the tambours I had at my command I only succeeded in recording 50 vibrations per second well, the 100 per second record being unsatisfactory. With the smallest bellows-recorder I succeeded in recording 50 vibrations per second easily, and 100 vibrations per second after a few preliminary trials. I reproduce two of these tracings in Fig. 10.

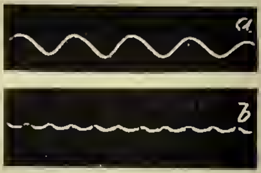

Fig. 10.

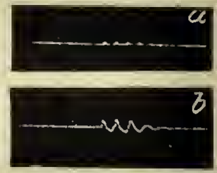

Fig. 11.

Fig. 10. Records of the vibrations of two tuning-forks transmitted from a tambour to the smallest bellows. In *a* the rate of vibration was 50 per sec. In *b* 100 per sec. The drum was rotated by hand.

In my hands the piston-recorder failed completely to respond to the 50 per second vibrations.

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[1] Marey. *Travaux de Lab.*, p. 38. 1875.

Another test of sensitiveness to minute changes consists in dropping a small weight from a known height on to a transmitting tambour connected to the recorder. This test was employed by Hürthle[1] in comparing the piston-recorder with a sensitive tambour, and the results are given in his paper upon recording by air-transmission. He allowed a weight of 0·2 gr. to fall 5 mm. upon the central disc of a small tambour. In employing this method I have slightly modified the test in that the weight I used was only 0·105 gr. which fell 5 mm. on to the centre of a thin rubber membrane 0·2 mm. thick stretched across a glass tube 43 mm. in diameter. No disc was fitted to the membrane. Fig. 11 gives the result of the two experiments, tracing (*a*) is that given by the piston-recorder[2], and tracing (*b*) by the smallest bellows-recorder. In both cases the magnification was eight-fold. The extreme sensitiveness of the bellows is well brought out in this tracing; each impact of a shot is clearly indicated by a very decisive wave and the rebound of the membrane is seen in the parts of the curves below the abscissa line. In other respects the curves speak for themselves.

As a result of these and many other tests of a somewhat similar kind, I conclude that the bellows-recorder is quite reliable as a volume-recorder. In all points of sensitiveness and rapidity of action it compares very favourably with the piston-recorder, which it excels in one direction, viz. that of range. It possesses the further advantage that it can be made of very large capacity without materially increasing the weight of the moving parts, and in these large forms it is quite as accurate as in the smaller. Moreover the weight of the moving parts in the bellows-recorder is considerably less than that of the similar parts in a piston-recorder of the same capacity.

I would especially emphasise the following points, which should be observed in recording volume by air-transmission.

(1) When details of rapid movements are required the tubing leading from transmitter to recorder should be of not less than 10 mm. in diameter, and should be as short as possible.

(2) The more extensive the air-movements the larger should the tubing be. Thus to record the tidal air in respiration in a cat, tubing of 20 mm. diameter should be used.

(3) In recording rapid movements of any amplitude the writing-point should not magnify the movement of the recorder more than five-fold, and the writing-lever must be tested to show that it is really rigid in the direction of the movement.

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[1] Hürthle. *Pflüger's Archiv*, LIII. p. 306. 1893.

[2] The piston-recorder has an internal diameter of 19 mm.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE LUNGS. PART I. THE BRONCHIAL MUSCLES, THEIR INNERVATION, AND THE ACTION OF DRUGS UPON THEM.

BY W. E. DIXON, M.D., *Assistant to the Downing Professor of Medicine in the University of Cambridge,* AND T. G. BRODIE, M.D., *Director of the Laboratories of the Royal College of Physicians, London, and the Royal College of Surgeons, England.* (Forty-nine Figures in Text.)

(*From the Research Laboratories of the Royal Colleges of Physicians and Surgeons, London.*)

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THE experiments we discuss in the present paper have arisen from some work we were conducting upon the circulation in the lungs. In this latter we found it possible to enclose a lobe of the lung in a plethysmograph, and it then became necessary to discriminate between volume changes due to blood on the one hand and air on the other. We therefore studied the movements of air in and out of the lobe; a study we were all the more induced to extend as there were many points concerned with the actions of the bronchial muscles and with the distensibility of the pulmonary alveoli that still required investigation. The present communication deals with the bronchial muscles alone, and we defer to later papers descriptions of our results upon the circulation in the lungs and the distensibility of the alveoli.

SECTION 1. EXPERIMENTAL METHODS.

Our aim in the experiments we describe in this paper has been to determine the volume changes of the lung or a lobe of the lung during its movements in normal and artificial respiration. The main number have been concerned with the study of a single lobe, which we have enclosed in oncometers whose general shape is seen in Fig. 1. These were made of gutta-percha, or in the later experiments of sheet copper, which possessed the advantage of lightness and rigidity. They were provided with a wide flange and were thus easily made air-tight by a glass cover. We have usually operated on the right lung because this lung is more subdivided into lobes than the left. The operation consisted in resecting four or five of the lower ribs—usually the fifth to eighth inclusive—until the lung was sufficiently exposed. The animal was then laid upon its left side and one of the lobes completely freed from its attachments down to the root of the lung. In the cat we have generally found it best to take the larger of the two lower lobes. This is attached by a fold of pleura along its inner edge to the tissues of the posterior mediastinum; when this is cut through the lobe is left free with a short and narrow pedicle. As the pedicle of the lobe is very short we have found it best to have no flange to the orifice of

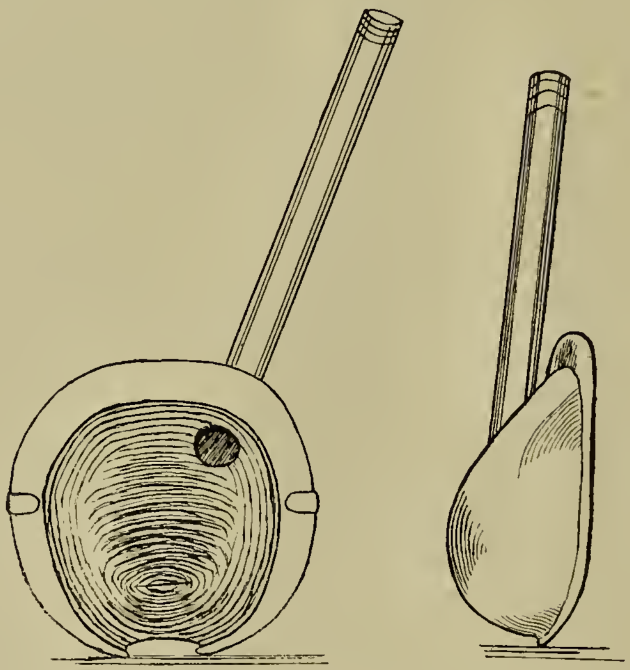

Fig. 1. Front and side views of the metal lung oncometer.

the oncometer. To make the orifice air-tight when the lobe is in position we use vaseline alone, adding "vaseline-cotton-wool" in those cases only in which the neck of the lobe was much smaller than the orifice of the oncometer. By using vaseline only we avoided any risk of unnecessary pressure upon the structures in the neck of the lobe.

To record the variations in volume we employed either a tambour, a piston-recorder, or the bellows-recorder recently described by one of us[1]; of these the two latter were chiefly used because they do not set up pressure variations in the oncometer. The piston-recorder employed was the latest pattern of the Hürthle model. This instrument has however the great disadvantage that it possesses a very limited range, one quite insufficient for many of our experiments. Later we had the bellows-recorder at our disposal and therefore chiefly used it.

In the preparation of the lobe for insertion into the oncometer we proceeded in one of two ways:—either great care was taken in the handling of the lobe not to compress it in any way, or else by gentle pressure the air was largely expelled from the alveoli. The latter method was the one we usually employed in our earlier experiments; in a lobe compressed in this manner a considerable pressure of air is required to again distend it to its normal inflation. If therefore, during artificial respiration, we employed a pressure just sufficient to produce a normal expansion of the other lobes we only obtained a partial expansion of the lobe experimented upon. By carrying the preliminary compression of the lobe one step further a condition is reached in which practically no air enters this lobe, provided the air pressure employed for respiration is kept so low that it is only just sufficient to inflate the rest of the lungs to a normal extent. By allowing free entrance and exit into the lobe, as in our later experiments, in which we used the bellows-recorder, we were of course studying the condition of the lung under much more normal conditions.

We have used two further methods for recording the volume changes due to the movements of the air in respiration. In the first of these an intercostal space was exposed and a small opening made into the thorax. A flanged glass tube was then introduced into the incision and fixed tightly in position by stitches. It is easy in this way to make the joint perfectly air-tight for low pressures. The tube was now connected to a bellows-recorder, which thus registered the amount of air driven out of the thorax at each inflation of the lungs. The walls of the thorax thus act as an oncometer, but they are not completely motionless. To keep them as nearly as possible at rest we therefore bandaged the whole of the abdomen and thorax fairly tightly. If, when fixing the glass tube in the thorax, it is tied in position about 4 cms. from the sternum and in the sixth interspace, this method makes a very simple though not very accurate experiment for studying the air volume changes of the lungs in artificial respiration. Care must, however, be taken that the lungs are not so far distended that the edge of a lobe is driven up to cover the orifice of the tube and thus form a valve.

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[1] Brodie. This *Journal*, XXVII. p. 473. 1902.

The other method employed consisted in tying a cannula in the trachea and then enclosing the animal in an air-tight box, through one wall of which the tube from the tracheal cannula is led. This is essentially the method of Knoll, modified however in that, by the use of a large bellows-recorder instead of a tambour, volume-measurements were taken instead of pressure changes. In these experiments either the animal was allowed to breathe normally, or respiration was carried on artificially. The latter, for many purposes, is more advantageous on account of the greater regularity of the movements, and because the experiment is then under better control. In several of the experiments of this type we have avoided the error due to any resistance to movement offered by the thorax and abdominal walls by resecting the whole of the sternum and the anterior third of the ribs on each side.

In addition to recording the volume variations of a lobe, or of the whole lung, we have in many cases simultaneously recorded the lateral tracheal pressure. For this purpose a T-tube was tied into the trachea, the lateral branch connected to a tambour covered with rather thick rubber membrane, and the other limb to the ordinary tracheal cannula and respiration pump. The tambour used indicated for each millimetre rise of the writing-point a rise of pressure of 6 mm. of water.

With regard to these records of tracheal pressure we must point out, that the method we used for the artificial respiration is by no means the correct one if we wish to record marked changes in lateral pressure for any variation in the bronchial resistance, for the air space between the respiration pump and the trachea was far too great. We have in nearly all our experiments purposely chosen it so, because we wished to keep the tracheal pressure changes as nearly uniform as possible throughout the experiment. Most of our tracheal pressure records therefore show no or only minimal changes, and consequently are not reproduced in our figures. It was only when working upon big animals and employing a large blast of air, with a relatively small lateral orifice in the tracheal cannula, that the variations in tracheal pressure became important. When in the few instances we have wished to bring about tracheal pressure changes following changes in the bronchial resistance, we have been easily able to do so by diminishing the thrust of the air-pump and nearly closing the lateral orifice of the tracheal cannula. In such cases we have taken care to allow sufficient time for the lungs to empty themselves through this restricted orifice.

The respiration pump and accessories. In any volumetric experiments upon the lungs one must be particularly careful that the successive respirations are completely uniform. In most of our experiments we used the method of inflating the lung by a blast of air, excess of which is allowed to escape through a side opening in the tracheal cannula. The form of respiration pump used consists of a large piston moving in a cylinder[1]. The piston was worked by a crank attached to a shafting which could be driven at any desired rate. The attachment of the piston-rod to the crank can be changed quickly, so as to alter the amount of air delivered at each thrust. The valves directing the course of the air work easily, but are perfectly air-tight. The upper end of the cylinder is closed in and provided with an inlet tube, so that any gas required can be administered in place of air. On the delivery tube is a specially constructed tap[2], by means of which any desired fraction of the air delivered at each thrust can be diverted into a small bottle containing an anæsthetic. In this way the amount of anæsthetic may be accurately graduated without in any way altering the total volume of air delivered to the tracheal tube at each inflation.

We did not find it any great advantage to moisten the air injected into the lungs, but we consider it of essential importance that it should be efficiently warmed. Several attempts to do this were made, such as by passing the air through several feet of lead piping immersed in hot water, but the ultimate and only really successful method of warming was as follows:—The delivery tube from the respiration pump was connected to the lower end of a metal cylinder fixed beneath the operating table. Inside the cylinder two incandescent eight candle-power lamps were fitted, and from the upper end of the cylinder another piece of tubing carried the warmed air to the animal. The connecting tube from the warming cylinder to the tracheal tube was kept as short as possible. The whole of the tubing from the pump to the tracheal tube was of large diameter. By means of this apparatus we could modify the pressure at which the air was delivered to the lungs in one of three ways, viz. (1) by altering the size of the exit from the tracheal tube, (2) by changing the amount of air delivered by the pump without altering its rate, or (3) by altering the rate of the pump. Throughout our experiments we have kept the pressure as low as possible, only allowing it to rise to just the necessary height for the efficient distension of the lungs.

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[1] This form of respiration pump is made from the designs of one of us (T.G.B.) by Mr C. F. Palmer, Brixton.

[2] Figured and described in the "Proceedings of the Physiological Society," p. xxxii. This *Journal*, xxvii. 1902.

Animals, anæsthesia, etc. The animals mainly used were cats, but the results were controlled by a considerable number of experiments on dogs and rabbits. With the exception of a few experiments, in which the animals were killed by pithing, all were anæsthetised, usually with ether, but at times with chloroform, or with the A.C.E. mixture. In experiments on dogs we generally administered morphia as well. In a few cases we anæsthetised first with ether, and then injected urethane either intravenously or intraperitoneally.

Care was always taken to keep the animal warm. Thus the room in which the operation was performed was always well warmed, and the animal lay upon a bed of cotton-wool supported on a copper box filled with water a little above body temperature. After the operation was ended the animal was covered up with cotton-wool.

In all the experiments an ordinary du Bois coil was used for excitation with a single Leclanché cell. The current could just be felt on the tongue when the secondary coil was at 14 cms. from the primary.

SECTION 2. INTERPRETATION OF THE RECORDS.

We reproduce in Fig. 2 a tracing[1] obtained in a typical experiment in which the lobe was distended at each inflation to approximately the normal extent. It clearly records simultaneously two variations in volume. The wave of large amplitude, *i.e.* each high vertical, is due to the movement of the air in and out of the lobe, and that of small amplitude is the blood-volume pulse. Unless otherwise stated the rise of the lever in all our tracings indicates increase in volume, *e.g.* inflation with air or increase in amount of blood contained within the lobe. An increase in the average amount of blood is represented by a rise in the line joining the bases of the waves, and conversely a rise of this line commonly, but not always, indicates increase in blood-volume. In order to show the various features of this tracing more clearly the artificial respiration was stopped during the part *a*. This brings out two points, namely, the amplitude of the blood-volume pulse and the residual air volume of the lobe. The gradual fall of the tracing after the cessation of the respiration shows that the lobe was nearly able to empty itself completely at each retraction. The amplitude of the volume-pulse (2·2 mm.) is seen to be very small in comparison with that of the air-wave (50 mm.). When the artificial respiration was restarted the amount of distension effected progressively increased for the first four inflations, when its original amplitude was regained. On

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[1] All the tracings in this paper are to be read from left to right. In all cases the time record gives seconds. All the tracings reproduced are from the right lung. L.V. stands for lung volume, B.P. for blood-pressure and T.P. for tracheal pressure.

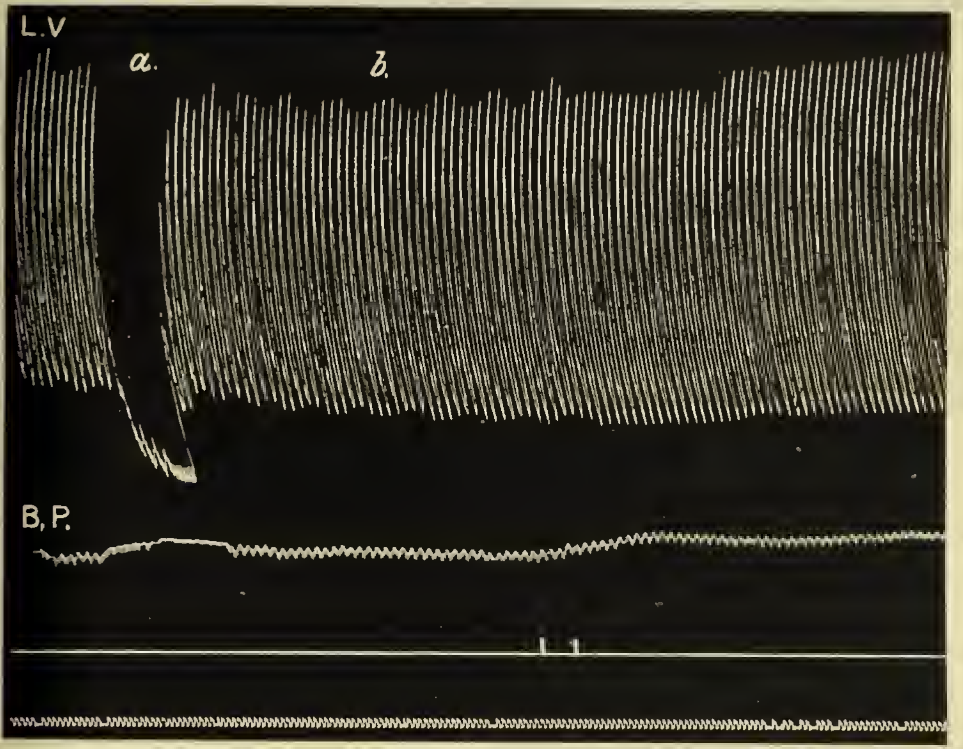

Fig. 2. Cat. Urethane and curare. One milligramme of muscarine previously administered. The zero B.P. is 16 mm. below the abscissa line. Reduced $\frac{1}{3}$.

account of the slow rate of the recording surface the air-waves appear as almost vertical lines with pointed apex and base. This is so in most of our tracings, for usually we were only desirous of studying the height of the wave, not its general form[1].

There are two lines in our curves to which we shall have to refer frequently and to which we therefore give names for convenience of reference. The first of these is that drawn through the apices of the air-waves and forms the upper border of the volume record. The second is a similar line drawn through the bases, and is the lower border of the record. We will term these the upper and lower limiting lines respectively. In Fig. 2 it is seen that there are waves in both these lines, those in the upper line being the more marked. This is generally true for all our tracings. The significance of these waves is discussed in the next section.

At about the middle of the tracing, at the instant indicated on the signal line the vagus was first ligatured, then cut. This changes the character of the tracing materially, the amplitude of the air-waves increases, and the waves on the limiting lines are completely altered. These changes are again referred to later.

With regard to the air movements there are three possible variations to study, (1) a change in the total amplitude, (2) a change in the rate of rise and fall, and (3) an alteration in the amount of residual air left in the lobe at the commencement of each inflation. The consideration of the second of these factors is deferred to section 8 of this paper. An alteration in the total amplitude of the air-wave, *i.e.* in the volume of air entering and leaving the lobe at each respiratory cycle, may be brought about by an alteration of one of three factors, viz., (1) a change in the air supply, either in the pressure or in the time during which it is allowed to distend the lobe, (2) an alteration in the distensibility and elasticity of the alveolar wall, or (3) an alteration in the resistance between the trachea and the alveoli :

(1.) The first of these is as a rule under direct control, and it is possible to so arrange the apparatus for artificial respiration that an alteration in the other two will produce so small an effect upon the first that it may be nearly always neglected. An instance in which this is not the case is recorded in Fig. 24, p. 136. In our experiments we have as far as possible avoided any error arising from this cause by using a large volume of air for each inflation, and keeping the tubing etc., leading from the pump to the trachea of large calibre, with a large lateral orifice in the tracheal cannula.

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[1] This latter we discuss in section 8 of this paper (p. 147).

(2.) The second factor can only be due, in our experiments, either to a change in the amount of blood contained within the pulmonary capillaries, or to the production of an effusion or secretion in this position. In all the changes we wish to discuss in this paper, effects of this kind either do not occur or are so minute that they may be neglected in face of the very large variations otherwise produced. That we are not dealing with any important change in the distensibility of the alveolar walls is easily determined by allowing a large volume of air at known pressure to distend the lobe before and after any particular observation. We have thus shown that the alveolus ultimately dilates to the same volume under the two given conditions.

(3.) The third factor in these experiments can only come into play in consequence of a change in the diameter of the lumen of the bronchioles. This diminution may be due either to muscular contraction of their walls or to swelling of the mucous membrane, but almost invariably the change has been muscular.

The evidence to be looked for as affording proof of contraction is a change in one of three factors:— (*a*) in the amplitude of the air-waves, (*b*) in an approximation of the limiting lines, or (*c*) a change in the rate of ascent or descent of the air-waves. The production of a constriction may be made to show itself in a simultaneous change of all these three, by altering the rate and force of the artificial respiration so that the air-waves are well marked and possess pointed apices. This latter point indicates that air was entering the alveoli during the whole of the inflation period. A flat-topped curve on the other hand indicates that the pressure of inflation lasted a longer time than was necessary to distend the alveoli to that pressure. In the sensitive state therefore a rise in the resistance of the air passages will be shown by a diminution in the rate of filling, and as the pressure only acts for the same length of time as before, by a diminution in the amplitude and a consequent alteration in the level of the limiting lines. The other method of experiment in which attention is directed to the rate of filling, and not to the total amount of distension, is not so convenient for experiment, and the occasions on which it is of special use are therefore given separately in section 8. A further convenient evidence of bronchial constriction is a rise of tracheal pressure, and we have consequently recorded it simultaneously in many experiments. Experiments of this class succeed easily when the rate of filling of the alveoli is relatively slow. This latter effect was brought about by using a lobe only partially distended. As previously pointed out gentle handling of a lobe produces a degree of collapse such that, while the remainder of the lungs is normally distended by the respiration pump, this lobe only dilates to about one-half or even less of the extent to which the others are distending. The reasons of this behaviour are by no means easy to determine. One possible explanation is that the compression of the lobe has completely emptied many of the alveoli, and the adhesion of their surfaces cannot be overcome by a pressure of inflation sufficient to fully distend a normal lobe. This condition would be accentuated by the action of the collapsed alveoli in restraining the free movement of the normal alveoli in their immediate neighbourhood.

The alterations in the direction of the limiting lines produced by a constriction may vary considerably, thus we may find rise or fall of either of the lines in all their possible combinations compatible with a diminution in the height of the air-waves. The most typical change is a rise in the lower and a fall in the upper limiting line. These may be of equal extents or much more marked in one than in the other. A common result is a very marked rise in the lower limiting line and a smaller rise in the upper, Fig. 17, p. 128. As to what will happen in any given case depends upon the following factors, (1) the degree of constriction, (2) the force of inflation, (3) the time intervals between successive inflations. By properly modifying the last factor one can accentuate at will any of the possible variations.

The interpretation to be placed upon the changes of level of these lines must be based upon the following considerations. With any given condition of the bronchioles the degree of inflation and its rate depend upon the duration and extent of the change in tracheal pressure. The corresponding features of deflation are due to an elastic recoil of the alveoli—a force which is small in comparison to the force employed in distension. Consequently, the rate of emptying is rather slower than the rate of filling, especially in the later stages. In an experiment arranged with the lobe in a sensitive state, the lobe is filling the whole duration of the inspiratory phase, and emptying the whole duration of the expiratory phase. If the high tracheal pressure at the end of inflation lasted longer the lobe would continue to expand, and also if the expiratory phase lasted longer the lobe would continue to contract. If now a constriction of the bronchioles be produced the rate of filling falls off, hence the height of the air-wave is less. The rate of emptying also decreases and consequently the fall of the lever is less. There is thus a tendency for the upper limiting line to fall, and the lower to rise. If the expiration time is further shortened we tend to produce a more marked rise in the lower limiting line and conversely. The greater the rise thus induced in the lower limiting line the less becomes the fall in the upper, until at last a stage is reached in which the upper line begins to rise. By still further continuing to shorten the expiratory phase a very considerable rise of this line, indicating marked over-distension, may be produced.

If on the other hand with fixed conditions of inflation and deflation a similar series of changes be produced they will indicate a gradually increasing change in the other main factor, viz., the constriction. The more marked the tendency to the production of over-distension becomes, the greater must be the constriction, but there is obviously yet another reaction possible, viz., one in which the constriction comes on so quickly and so forcibly that no time is allowed for over-distension, and collapse of the lobe is produced with complete absence of air movements.

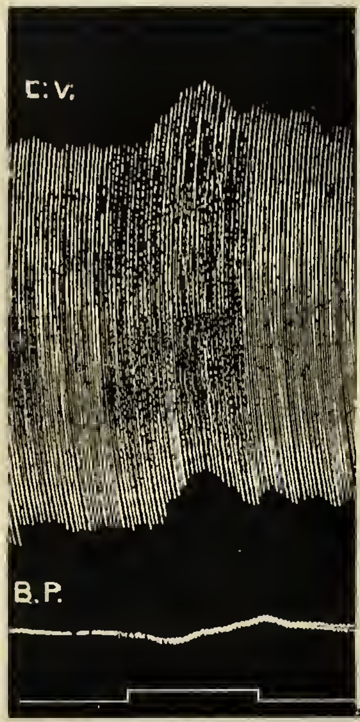
To recapitulate the main conclusions: it has been shown that in the employment of our method a constriction of the bronchioles is proven by one or more of the following changes in the tracing:

1. A diminution in the amplitude of the air-waves.
2. An alteration in the rate of rise and fall of those waves (especially the latter).
3. The production of an increased and permanent inflation, *i.e.* a rise of the lower limiting line.

SECTION 3. THE CAUSE OF THE WAVES SEEN IN THE LIMITING LINES.

The waves which occur on the limiting lines (cp. *supra*) are of two varieties. In the first of these the crest and trough of the waves respectively correspond in the upper and lower lines; when this happens the two waves are always of the same amplitude and consequently the total amplitude of the whole tracing does not vary. These waves are relatively rare, and when present are usually rather irregular. We reproduce in Fig. 3 a tracing showing this form. They can only be produced in one of two ways. The first and more usual is a rhythmic variation in the total blood-volume of the lobe, and frequently synchronous variations in the aortic pressure curve are seen. The second possibility, which must always be excluded, is that the lobe was not properly fixed in the oncometer and that rhythmic movements on the part of the animal (*e.g.*, respiratory) pulled upon the root of the lobe. Waves of this origin are of course excluded by the use of curare.

The second variety of waves is the more common : here the crest of the wave in the one line corresponds in time to the trough of the wave in the other. The waves in the tracing of Fig. 2 belong to this variety. Waves of this form may also be produced in one of two ways. (*a*) By variations in the tonus of the bronchial muscles, or (*b*) By interference effects between the air and the blood-volume.



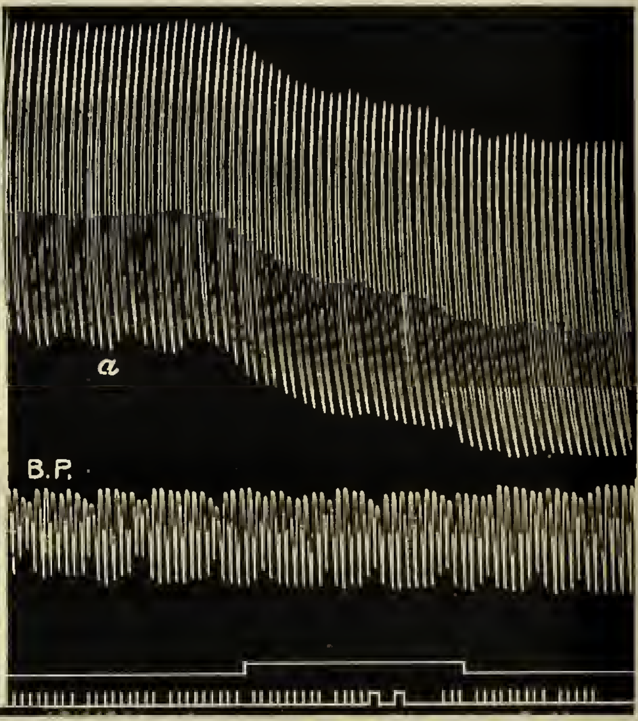


Fig. 3. Cat. A.C.E. The zero B.P. is 38 mm. below the abscissa line. During the interval indicated by the signal the vagus was first ligatured and then cut. Reduced $\frac{1}{3}$.

Fig. 4. Dog killed by pithing. Upper tracing tracheal pressure. During the time indicated by the signal the artery to the lobe was clamped. Reduced $\frac{1}{3}$.

We have never observed tonus waves in any of our tracings at the commencement of an experiment, *i.e.*, under normal conditions. They only made their appearance as the result of some action, such as vagal stimulation or the use of a drug. Thus in Fig. 20, p. 131, waves are produced by stimulation of the vagus. In Fig. 36, p. 158, a dose of pilocarpine is seen to produce a very marked wave of constriction before completely occluding the bronchioles to the lobe.

But the waves usually seen are interference in character, as may be proved by temporarily arresting either of the two factors producing them. Thus in Fig. 2, where at *a* the air movements were stopped, the waves disappeared. The three small waves seen just after the tracheal tube was disconnected from the respiration pump are due to accidentally allowing a little of the air blown from the pump to enter the open end of the tracheal tube. If, on the other hand, the blood be excluded from the lobe the interference waves again disappear and we get air-waves of uniform height. This can be done by suddenly clamping the vessels running to the lobe, or as in Fig. 4 by clamping the artery only. In this experiment the artery was clamped during the time indicated on the signal line, and was released at the instant the signal fell, but did not at once fill again, for the lobe was seen to remain bloodless. The waves in the first part of this figure are most marked in the lower limiting line. The fall in this line directly after clamping the artery is due to the emptying of the lobe of blood, the vein having been left untouched. The irregularities in the descending part of the same line are due to slight movements of the clamp pulling upon the lobe: they correspond exactly with similar variations on the upper limiting line. By excluding the blood therefore the waves have again been abolished.

Apart from demonstrations of this type, which are not always possible, we are able to prove from examination of the tracing itself that we are dealing with interference effects, by applying the known tests for waves produced by interference. Thus, if the number of respirations and heart-beats which go to form a complete cycle be counted, it is found that the number of heart-beats + or – 1 is an exact multiple of the number of respirations. Thus for the wave marked *a* in Fig. 4 the number of respirations is 7, and the number of heart-beats 8. Again, for the wave marked *b* in Fig. 2 the number of heart-beats is 31, and of respirations 6.

Yet a further proof exists in that the sum of the total amplitudes of the waves on the limiting lines is double the amplitude of the blood-volume pulse. Thus in Fig. 2 the amplitude of the wave on the upper limiting line is 3 mm., that on the lower line is 2 mm., and the volume-pulse is 2·5 mm. A similar result has been obtained by us in nearly all the waves which so frequently occur throughout our tracings. These waves are always most marked when the lobe is only being partially expanded, a result to expect from *a priori* reasoning, for then the ratio of the volume-pulse to the air-wave is greater. Further, the waves are very variable in length, rarely continuing of the same length for long. This is because the heart rate is continually altering while the respiratory rate remains constant. This variability is well seen in Fig. 2, and is again exemplified in Fig. 5. For the wave *a* of this

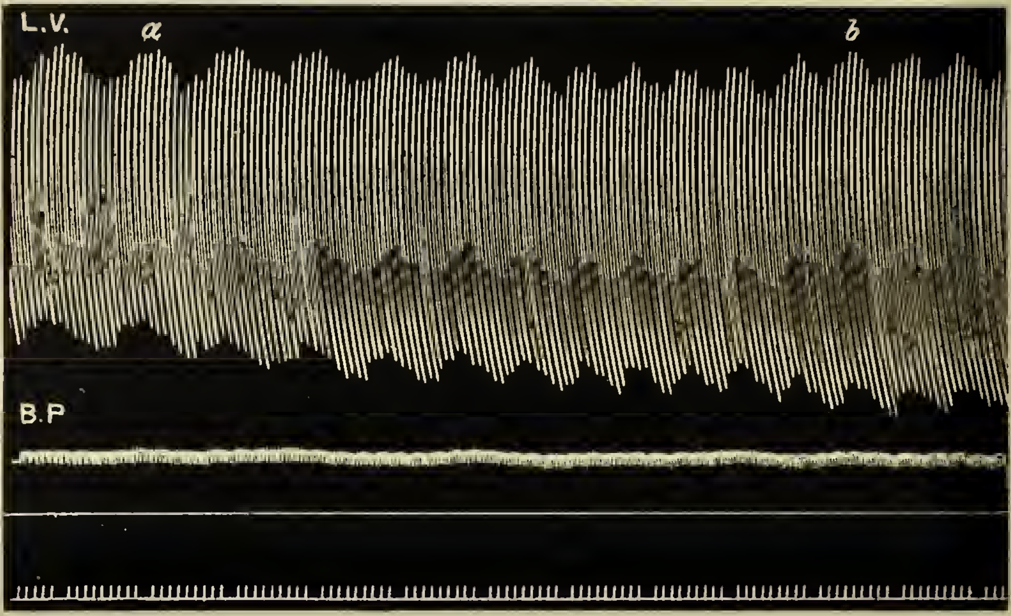


Fig. 5. Cat. A.C.E. R. vagus cut. The animal had previously received 0·01 grm. pilocarpine. Zero B.P. lies 60 mm. below the abscissa. Reduced $\frac{1}{3}$.

tracing the number of inflations is 12, and of heart-beats 25. For the wave *b* the numbers are 8 and 17 respectively, the time interval between the two being only 90 seconds. The difference is due to an

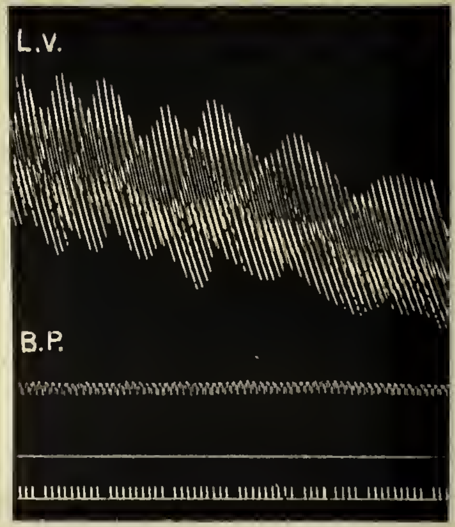


Fig. 6. Cat. A.C.E. Zero B.P. lies 21 mm. below the abscissa. Reduced $\frac{1}{3}$.

alteration in the rate of the heart-beat, which at *a* is 32 in 10 sec., while at *b* it is 34 in the same time.

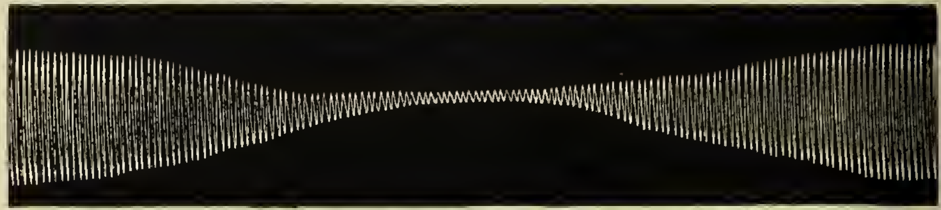
Another feature of the tracings is that the notches on the air-waves produced by the volume-pulse fall successively later or earlier, as the case may be, upon the air-wave, and that the notch thus appears to travel either up or down the air-wave culminating at a crest or trough of the waves on the limiting lines. This is seen in Fig. 2, but we give another instance in Fig. 6, as it illustrates the point far better. In this figure the wave *a* is produced by 12 respirations, and 37 heart-beats.

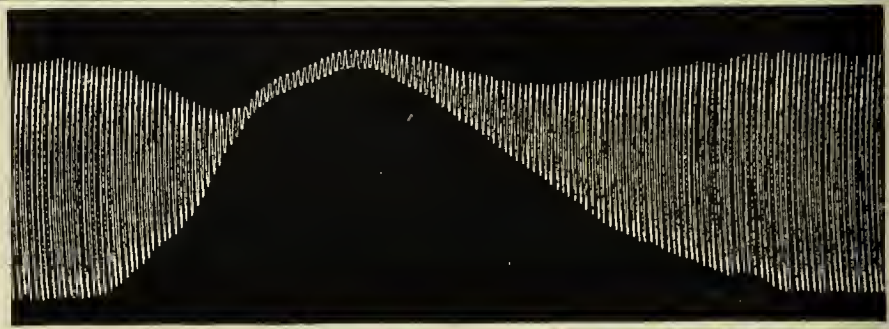
To sum up, waves are most commonly due to interference, and their mode of production is exactly comparable to the phenomenon of beats in sound waves. They may also be due to rhythmic contractions of the bronchial muscles; thus we have observed them immediately after the death of an animal, *i.e.*, after the heart has ceased to beat (Fig. 34, p. 154), and in artificially perfused lungs, where the pressure of perfusion was maintained at a constant height.

SECTION 4. SOME EXPERIMENTS ON A SCHEMATIC LUNG.

In order to exemplify and control some of the results obtained upon the lung we conducted a few experiments upon an artificial pulmonary alveolus represented by an elastic bag. This was enclosed in a bottle and its orifice tied upon a short piece of glass tubing passing through the rubber cork of the bottle. A second glass tube through this cork was connected to a bellows-recorder, which thus registered the volume variations of the sac, the bottle acting as an oncometer. The rubber bag was now distended rhythmically by the respiration pump, to which it was connected through a tracheal cannula. Between the cannula and the bag was a length of rubber tubing provided with a screw-clip. The rubber tubing thus represented the muscular-walled bronchus, the lumen of which could be restricted by the screw-clamp.

In the next two Figures are given the results of experiments in which the tube leading to the balloon was very gradually constricted and then as gradually released. The effect upon the volume per inflation commences directly the constriction begins, progressively increases with it, and falls off as the constriction is relaxed. Both figures show a diminution of the amplitude of the air-wave as the constriction is increased, but the other features of the tracing are markedly different. In Fig. 7 the upper limiting line falls and the lower rises, whereas in Fig. 8 the upper line falls a little at first, then quickly rises, while the lower rises throughout. In both we get a reversal of the changes as the constriction is decreased, and both show





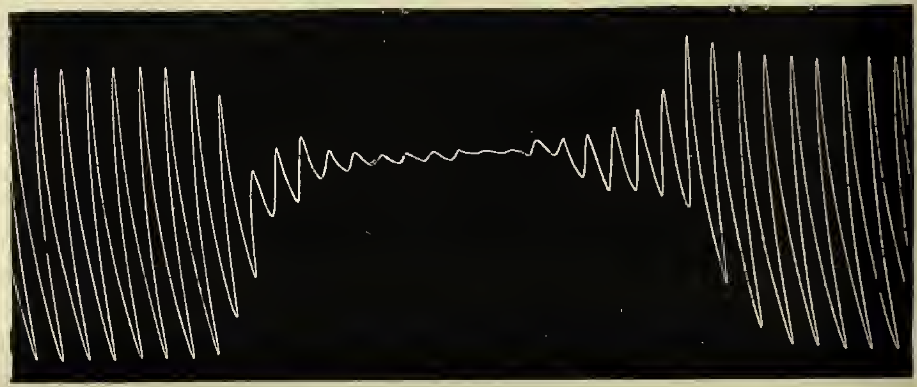


Fig. 7. Tracing obtained from a thin-walled rubber sac rhythmically distended as in artificial respiration. Rate of pumping was 3 in 2 seconds. The inlet tube to the sac was gradually constricted and gradually relaxed.

Fig. 8. Similar tracing to that of Fig. 7. The only change is in the rate of pumping, which now is 1 per second. The rate of movement of the drum has been proportionately changed.

Fig. 9. Similar tracing to the two preceding. The only change is in the rate of pumping, which is now 1 distension in 4·5 secs.

that as the constriction was produced, less and less air was forced through the resistance into the balloon. The variations in the limiting lines illustrate the points already discussed on pp. 106, 107; and the way in which the two effects were produced are therefore of interest in this connection. Both tracings were taken from the same bag within a short time of one another, the only difference in the experiments being that in the second (Fig. 8) the rate of pumping was decreased to two-thirds the rate used in the first, the rate of movement of the drum being proportionally altered. The maximum pressure of inflation was therefore decreased, but, as measurement showed, only to a slight degree. In spite of the greater maximum tracheal pressure attained during each inflation the curve is of less height, for the variation in height is rather a function of the time-duration of the rise of pressure—in this instance being very nearly directly proportional. Thus the ratio of the times is as 2 : 3, and the heights are 21 and 32 mm. respectively. A further difference, which however is unrecorded in the Figures, is that the balloon during Fig. 8 was more distended at the end of deflation than in Fig. 7. The reason there is such marked over-distension in the second case and not in the first is therefore clearly due to the relatively greater difficulty of emptying as compared to filling. This greater difficulty is due to two causes, first, because there is more air to drive out, and secondly, because the internal pressure of a thin-walled rubber balloon falls the more it is distended. This is patent from the well-known fact that if two exactly similar balloons, one distended considerably, the other to a less extent, be connected to one another, the less distended one empties itself into the more distended. Whether we have a similar phenomenon at play in the movements of the pulmonary alveoli is a point to which we return later.

It is also possible to convert the first effect of the two preceding figures into the second in yet another way, viz. by increasing the distending pressure so that the movements of the balloon are more marked. In this manner again we attain the same end, that is, the balloon is made to empty a larger volume of air each phase. The emptying being effected by a smaller force than the filling, it is consequently more influenced by an increased resistance, so that as this latter grows the over-distension becomes progressively more marked.

Both these reactions are sensitive in that they show well-marked variations following a small constriction of the inlet tube; they are also sensitive for another reason, not to be gathered from the figures themselves, viz. that the whole of the changes were brought about by a relatively small increase in the resistance, and that a distinct effect was produced immediately the resistance was increased. For comparison with these results we give a third in Fig. 9, which was obtained from the same balloon and shows how the sensitiveness has been lost by reducing the rate of the respiration pump. The surface was travelling at the same pace as in Fig. 8. The rate of the pump was 4·5 times slower, and the amount of air delivered each inflation was the same. The most important difference is that the change in amplitude does not commence when the constriction was begun. When it is produced it is very marked, and is sudden in onset, not because the rate of increase of the resistance was more sudden than in the preceding cases, but because a marked change could only be produced when the tube leading to the balloon was nearly occluded.

From these experiments on balloons we conclude that working with an artificial system designed in imitation of the lung, it is necessary, if we wish to obtain sensitiveness in the direction of being able to show small changes in the resistance of the tubes leading to the distensible sacs, to employ a force of inflation sufficiently great to give a good distension and worked fairly rapidly. These conditions we have also found to hold true for the lungs with certain reservations which are discussed when considering the action of the vagus on the bronchioles. We should mention that in addition to the two effects we have reproduced in Figs. 7 and 8, yet a third is possible and may be readily produced if desired. This is an entire absence of over-distension, *i.e.*, during the constriction the amplitude falls off as before but the lower limiting line of the tracing remains straight. This is a condition frequently observed in the lungs, and can be at once reproduced by allowing a sufficient time for the sac to completely empty itself at each deflation.

SECTION 5. BRONCHO-CONSTRICTOR NERVE-FIBRES.

The question of the innervation of the bronchial muscles has been the subject of many investigations, but it was not until the papers of Einthoven and of Beer appeared that the existence of constrictor fibres in the vagus was satisfactorily demonstrated. Our observations have been very numerous, extending over more than 100 animals, chiefly because we found so many negative or partially negative results that it was necessary to determine their meaning. One important result of our work is that we are able to satisfactorily demonstrate the existence of broncho-dilator fibres running side by side with the constrictor fibres (cf. p. 138). Their presence was the cause of many of the initial difficulties. In the description which follows we first give a short account of the previous literature, for which we are largely indebted to the papers of Gerlach, Einthoven, and Beer, and then proceed to our own experiments.

i. Historical.

Williams[1] was the first to investigate the action of the vagus upon the muscles of the trachea, bronchi, and bronchioles. His animals were killed, and a tube, tied tightly in the trachea, was connected to a water-manometer. The trachea and lungs were then removed and a large sheet electrode was fitted around the lower margins of the lungs. A current from a powerful battery was now passed through the lungs to the brass cannula in the trachea. Each time the current was closed the manometer rose considerably (50 mm.), only falling slightly if the current was kept closed. Williams found that though the fibres were easily fatigued the effect could be obtained repeatedly for about an hour after the death of the animal if intervals of rest were allowed. If the lungs were too distended only poor effects were obtained.

Mechanical excitation of the vagus was ineffective, as also was the passage of a constant current through the nerve, although the œsophagus was strongly 'convulsed' by this stimulation. On cutting into the lung he found that the bronchi contracted powerfully to mechanical excitation. He concluded therefore that the bronchial muscles were easily excitable directly, and that the vagus was not their motor nerve. His experiments give evidence which, in the light of our present knowledge, clearly indicate that by his method the bronchial muscles were really being excited indirectly, for he found that it was not possible to register a contraction later than about one hour after death, and further that a dose of strammonium or atropine given just before the death of the animal prevented the occurrence of the reaction. Under both conditions we now know that the muscles are still capable of contracting if suitably excited.

Longet[2] was the first to show that the vagus contained motor fibres to the bronchial muscles. He directly observed the bronchi, exposed by cutting through the lung, in freshly killed large animals (horse, ox), and found that they contracted when the vagus was excited electrically. He points out, however, that his experiments do not permit him to say whether the minute bronchi behave similarly.

Volkmann[3] also obtained positive evidence of the action of the vagus on the bronchi. His method consisted in tying a glass tube, drawn out to a fine nozzle, into the trachea. The animal was killed and a small flame placed in front of the nozzle. On stimulating the vagi he, in all cases, observed movements of the flame, and in one experiment it was even blown out. He obtained the same result when the thorax was opened, though the movements were then much weaker. Here again the results though positive give us no proof of the influence of the vagus upon the smaller bronchi. They only tell us that the air space contained within the lung has been decreased, but that might well be due entirely to an action upon the trachea and large bronchi. The same criticism must hold for any method that attacks the problem statically. Most of the early observers employed such a method, usually recording the intra-pulmonary pressure by a water-manometer connected to the trachea of a recently killed animal, after the manner employed by Williams. Thus Donders[1] in his classical work upon the force of retraction of the lung recorded the pressure in this manner, and although he investigated the effect of vagal stimulation on several occasions he invariably obtained negative results. A probable explanation of his lack of success may be that the lungs were too highly distended when the vagus was excited. Similarly Wintrich[2] obtained negative results, when the thorax was either opened or intact. Knaut[3] however obtained positive results in the greater number of cases, but the rise in pressure was in no case very great. The results of experiments of this class were criticised by Rügenberg[4], who ascribed the positive results to the movements of the œsophagus simultaneously produced.

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[1] Williams. *Brit. Assoc. Reports*, 1840, p. 411.

[2] Longet. *Comp. Rend.*, t. xv. p. 500. 1842.

[3] Volkmann. See Article "Nervenphysiologie" in *Wagner's Handwörterbuch der Physiologie*, II. p. 586. 1844.

Bert's[5] experiments were of the same type, but he introduced the important modification of recording the pressure changes by a tambour instead of by a water-manometer. At first his results tended to confirm Rügenberg, but he later found that the negative result was due to the fact that he was distending the lung to too high a pressure. When working upon collapsed lungs he always obtained positive results. As these experiments were performed upon dogs he pointed out that it was the vago-sympathetic that was excited, and to determine whether the active fibres belonged to the vagus or to the sympathetic, he tested the action of the vagus in reptiles and found it to cause contraction of the lungs both in the case of turtles and lizards. He further found that the right vagus in these animals innervated the right lung only, and conversely.

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[1] Donders. *Ztschr. rat. Med.* III. p. 292. 1853.

[2] Wintrich. *Virchow's Handbuch d. spec. Path. u. Ther.* v. Abt. I. p. 189. 1854.

[3] Knaut. *Dissert. Dorpat.* 1859.

[4] Rügenberg. *Studien des Physiol. Instit. zu Breslau.* Heft 2, p. 47. 1863.

[5] Bert. *Leçons sur la physiologie comparée de la respiration*, Paris, 1870, p. 369.

Schiff[1], accepting the proof that excitation of the vagus caused a contraction of the lung, attempted to determine whether the alveoli or the bronchi were the parts which contracted. For this purpose he removed the lungs with the trachea and vagi from an animal immediately after death. Having slightly distended the lungs the trachea was stopped by a rubber cork, on to which it was tied tightly. The lungs were then laid upon a table, the nerves placed across electrodes which could be worked from a distance, and a thin bright metal disc placed on the edge of a lobe. The edge of this disc was watched through a telescope from a distance. On stimulation of the vagus he obtained negative results in most cases, in five the disc fell a few fractions of a mm., and once it showed slight rotatory movements. He thence concluded that it was the parenchyma of the lung which contracted and not the bronchioles.

Though his experiments may be considered confirmatory to those showing that the lung contracts on vagal excitation, they certainly cannot be interpreted as discriminating between contraction of the bronchioles and contraction of the parenchyma. If the small bronchioles contract in a lung at rest they must pull upon the neighbouring parts, and so can lead to a decrease in thickness of a lobe, without necessarily altering the volume of the alveoli. His readings, moreover, indicated such minute changes that the method cannot be considered delicate enough for the purpose.

Toeplitz[2] returned again to observation of the intra-pulmonary pressure in recently killed animals but could only obtain negative results.

Gerlach[3] repeated these experiments, employing a water-manometer to record the change in pressure. Working upon excised lungs somewhat after the manner adopted by Toeplitz he always obtained negative results, whereas if the lungs were left *in situ* positive results were gained. Thus in a dog he obtained a rise of 6 mm. of water when both vagi were excited, and in a rabbit a rise of 4 mm. This success led him to work upon curarised animals. The pleuræ were opened and the

[1] M. Schiff. *Pflüger's Archiv*, IV. p. 226. 1871.

[2] Toeplitz. *Ueber d. Innerv. d. Bronchialmusculatur. Dissert.* Königsberg. 1873.

[3] L. Gerlach. *Pflüger's Archiv*, XIII. p. 491. 1876.

vagi excited during a pause in the artificial respiration. He again obtained positive evidence, but the rise of pressure recorded was very small, usually about 5 or 6 mm. of water. Though the use of living animals is an obvious advance it possesses two great disadvantages. In the first place, each heart-beat expresses itself upon the manometer and consequently makes it difficult to read with accuracy. Secondly, some or all of the pressure changes might be of vascular origin, a source of error which Gerlach avoided as far as possible by only employing weak stimuli. The latter point is of very serious importance where such small pressure changes are alone produced. Gerlach excluded all possible error which might arise from movements of the œsophagus or stomach or from contraction of the trachea. He concludes therefore that the effects are due to the bronchioles.

The experiments of Bókai[1] were practically of the same type. He excluded the influence of the trachea by employing a long cannula which he pushed down to the bifurcation. This was then connected to a tambour. The animal was killed, and the chest widely opened, the diaphragm cut away, the heart supported, the œsophagus cut through and the stomach removed. After these precautions he always obtained positive results when the vagi were excited, provided the lungs were not too fully distended. He found the left vagus gave better results than the right.

In more recent times Sandmann[2] working upon curarised animals, and recording the tracheal pressure by a tambour, only obtained slight changes. He excluded any error due to movements of the œsophagus as in Gerlach's experiments and gives curves showing a rise of pressure on excitation of the vagus, but, as in all experiments of this type, the changes are very slight. He states that many were negative and that often both positive and negative effects were obtained from the same animal. He employed atropine to exclude cardiac changes and stated that in some instances a dose just sufficient to paralyse the cardio-inhibitors left the bronchial fibres still active. In this we have completely failed to confirm him, but are in complete agreement with both Einthoven and Beer.

As we have already pointed out, experiments by this method are very unsatisfactory for several reasons. Thus, they do not distinguish between the bronchi and bronchioles, and the effects in the best of cases are quite minimal. It is consequently very difficult, if indeed possible, to exclude influences produced by circulatory changes. One reason why the recorded results are so small is probably because the air driven out of the bronchi during contraction need not necessarily lead to a rise of intrapulmonary pressure of any measurable extent, but may simply distend the alveoli and thus escape recognition. It is suggestive in this respect that all observers failed to obtain a rise if, at the start, the lung was moderately distended, for we know that the pressure difference required to start the distension of a collapsed lung is much higher than that required for an already distended lung.

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[1] A. Bókai. *Orvosi Hetilap,* No. 25. 1880. Abstracted in *Hofmann und Schwalbe's Jahresbericht,* IX. p. 79. 1881.

[2] G. Sandmann. *Arch. f.* (*Anat. u.*) *Physiol.* 1890, p. 252.

We must therefore conclude that a satisfactory method of demonstrating the variations in calibre of these small tubes must be dynamical, and one by which we can investigate the amount of air passing through the tubes or demonstrate a variation in the resistance offered by them. One of the first to realise this was MacGillavry[1], who pointed out that a contraction of the bronchioles in a collapsed lung could only produce a minute diminution of volume, and that the resulting rise of pressure, which is the factor measured in those cases in which the problem is studied statically, must of necessity be still less. The ingenious method he employed was to displace air from a large reservoir at a constant rate into the lung. The lung was well pricked over its surface to allow an escape of the air through the alveolar walls and the lateral tracheal pressure was then measured. He found that in one experiment the pressure rose from 70 to 100 mm. of water on exciting the left vagus, and from 70 to 85 mm. on exciting the right. He thus obtained quite conclusive evidence of the existence of broncho-constrictor fibres in the vagus, but stated that he always recorded better results with the left vagus than with the right, a difference he ascribes to a greater number of fibres in the left than in the right. We think it more probable however that the difference is due to his method of experimenting. A freer exit from the alveoli of the left lung for instance would satisfactorily explain the differences he observed. Unfortunately his method can only be applied satisfactorily to a recently killed animal, for when experimenting upon living curarised animals his results were by no means so decisive and the bleeding produced by the pricking materially obscured the interpretations to be placed upon the results.

Riegel and Edinger[2] give a good historical account of the previous work upon the subject. Their paper is especially valuable in that they record confirmatory experiments performed with the object of testing previous work. Thus they repeated and confirmed Donders and Gerlach, and confirmed MacGillavry, though they did not obtain quite so large a rise in pressure as he had obtained. They conclude therefore that there is no question that the vagi innervate the bronchial muscles, and the rest of their paper deals with the consideration whether the spasm of the bronchioles which can thus be obtained is sufficient, in itself alone, to explain all the symptoms observed in spasmodic asthma.

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[1] T. H. MacGillavry. *Arch. Néerlandaises des Sciences*, XII. p. 445. 1877.

[2] Riegel and Edinger. *Ztschr. klin. Med.* V. p. 413. 1882.

Roy and Graham Brown[1] investigated the subject by a totally different method, viz., by introducing a narrow glass tube into the trachea of a dog and passing it down until it reached about the middle of the lower lobe of the lung. The bronchial end of this tube was closed by a small oblong bladder of animal membrane, which was distended to fill the small bronchus, and the movements of the bladder were then recorded by an oncograph. The curves they obtained they describe as complex, but as the method only deals with rather large bronchi we need not describe them fully here. It is sufficient for our purpose to state that positive effects on vagus excitation were obtained, that atropine caused complete paralysis of the vagal endings, and that tonus was usually present, dilatation following section of the vagus.

Franck[2] has studied the effect of cortical excitation upon the bronchial muscles, his method consisting in recording the extent of the chest movements during artificial respiration in the curarised animal. He obtained constriction, abolished by section of the two vagi, on stimulating parts of the motor areas of the cortex. The figure he gives shows only a slight change, however, not sufficiently decisive to place the conclusion he draws from it beyond question.

Chauveau[3] states that in the horse the bronchioles show some tonus which disappears on section of the vagus, for he found that if the vagus be cut on one side the vesicular murmur on that side disappears and is weakened on the opposite side.

The method adopted by Lazarus[4] was to distend the lung from a small cylinder, into which water was run at a fixed rate, and record the rate of rise of pressure and the total rise; similarly expiration was effected by running water from a second cylinder into which the lungs were allowed to empty and recording the rate of fall of pressure. The figures given by Lazarus show changes very slight in amount and therefore open to the objection that they may be to a large extent of vascular origin. He experimented upon curarised rabbits, and obtained positive results on vagal excitation.

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[1] Roy and Brown. *Proc. Physiol. Soc.* p. xxi. This *Journal*, VI. 1885.

[2] Franck. *Fonctions motrices du cerveau.* Paris, 1887, p. 145.

[3] Chauveau. *Semaine med.* 1889, p. 117.

[4] J. Lazarus. *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 19.

Unquestionably the most important work upon the subject that has hitherto appeared is that of Einthoven[1], who attacked the problem by a totally different method. The experiments were conducted upon curarised and morphinised dogs, in which the distension of the lungs was effected rhythmically by injecting air from a syringe of moderate size into the trachea. The syringe was connected by a short length of tubing to the trachea so that the air space from the syringe down to the small bronchioles was practically only that of the upper air passages. It was worked rhythmically, and for a short time at the height of each distension the trachea was connected to a mercury manometer, which thus recorded the maximum pressure attained. A hindrance to the entrance of air into the alveoli means more air retained in the trachea and therefore a higher maximum pressure. By this method Einthoven was able to trace the course of the contraction with considerable accuracy, and if there is any criticism to be made of the excellent results he records it is that the method gives us no indication of the state of distension of the lung. He also gives one experiment in which the movements of the thorax were studied, and in that way shows how the movements of the lungs are limited by excitation of the vagi. The experiment thus recorded agrees in its general form very much with our tracing Fig. 11, p. 123.

In the same year Beer[2] published a long series of experiments in which the question of the action of the vagus was tested by many methods. In all cases he obtained evidence of the existence of broncho-constrictor fibres. We need not enter into a full discussion of his results, it is sufficient for our purpose to record the methods adopted. These were: (1) to record the intrapulmonary pressure in a curarised animal; in these, better results were obtained when the thorax was intact than after it had been opened. (2) To record the full variations of the lateral tracheal pressure in curarised animals in which artificial respiration was regularly maintained. (3) To record the rise in tracheal pressure (Expirationsstoss) produced by the emptying of the lungs into a box with a relatively large opening to the external air.

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[1] Einthoven. *Pflüger's Archiv*, LI. p. 367. 1892.

[2] Beer. *Arch. f.* (*Anat. u.*) *Physiol.* 1892, Suppl. p. 101

(4) To record the movements of the diaphragm in curarised animals. In these experiments, when carried out during an interval when the artificial respiration was stopped, Beer observed a movement downwards of the diaphragm, which he explains as being caused by the distension of the alveoli by some of the air driven out from the bronchioles. On the other hand when recording the diaphragm movements while artificial respiration was still continued, he observed diminution in the extent of the movements and a permanent lowering in expiration, indicating distension of the alveoli.

ii. Our own experiments.

A. *Those in which the lobe was normally distended during each respiration.*

The results we give are those which we consider the most typical. Great care was taken that the lobe experimented upon was uninjured, and that it moved quite freely in the oncometer. As showing certain differences we give tracings taken from the dog, rabbit and cat respectively. In all cases the aortic blood-pressure was simultaneously recorded.

Fig. 10 is from the lower lobe of the right lung of a small dog. The upper tracing is that of the tracheal pressure, the middle, the volumetric record of the lobe, and the lower is the aortic pressure. At the time indicated by the signal the right vagus was excited. The effect upon the blood-pressure is typical. The effect upon the lung volume is a very marked diminution in amplitude of the air-waves commencing at once and quickly attaining a maximum. The effect passes off slowly after cessation of excitation. The extent of the change is shown by the following figures. The height of the air-waves at *a*, *i.e.* before excitation, is 51 mm. The heights of the four waves immediately following the commencement of the excitation are 48, 45, 42 and 36 mm. respectively. At *b*, the height is 22 mm., at *c*, 37, and later, 44 mm. At the height of the constriction, therefore, only about two-fifths of the quantity of air enters, as compared to the stage before excitation. The maximum constriction occurs at the fourteenth inflation. Comparing this with the record of the lateral tracheal pressure, we see that this rises quickly and attains its maximum at the eighth inflation It then remains up during the excitation and falls gradually as the constriction passes off. This tracing indicates maximum pressures of 114 mm. of water at *a*, 192 at *b*, 159 at *c*, and 156 later. There is thus a distinct difference in time between the positions of the two maxima of constriction, as judged by the volume change on the one hand, or the pressure change on the other. It is difficult to say which gives the course of the bronchial contraction most accurately. A point against the lung volume record in this respect is, that the lung does not immediately come to a position of equilibrium for a sudden change in the conditions of inflation, but takes some three or four respirations to do so. (See Fig. 2 at *a*.) This delay is very little marked if the lung is being fully distended, but becomes more and more so the greater the collapse. For this reason we must not consider the volume changes as necessarily accurately indicating the relative amounts of constriction at different times. The volume change at once shows any change in calibre of the bronchioles and gives a direct measure of the effect of that change upon the lung. For the study of the course of the constriction, its amount and time relations, the pressure curve is possibly a nearer approximation than the lung volume curve. In this tracing recovery is at first quick, then slow, but never quite complete:

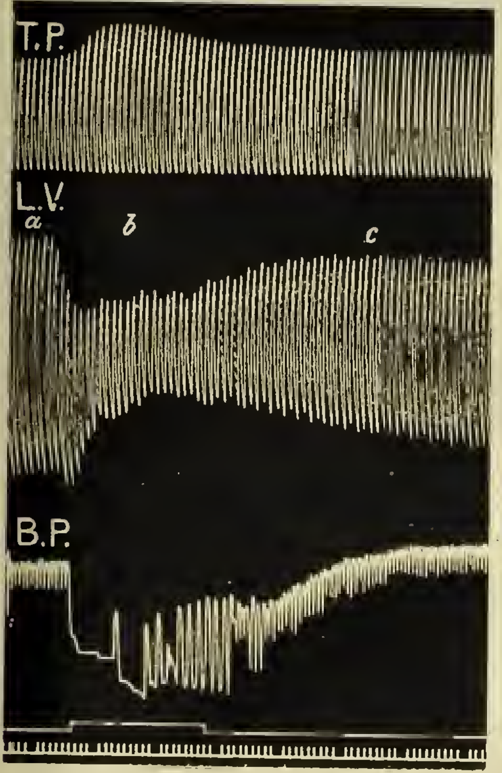


Fig. 10. Dog killed by pithing. Excitation of vagus; coil at 6 cms. Reduced $\frac{1}{2}$.

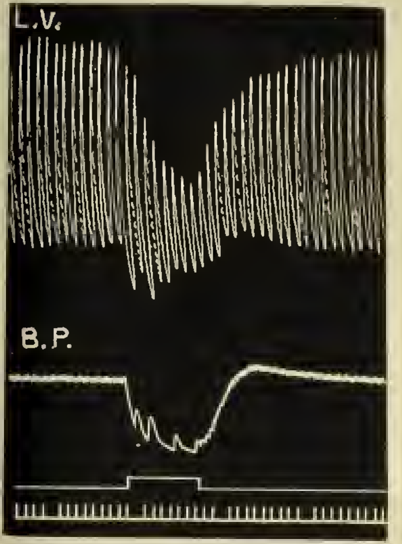


Fig. 11. Rabbit killed by pithing. Stimulation of vagus; coil at 6 cms. Reduced $\frac{1}{3}$.

this is a very common phenomenon and apparently an important feature of the physical properties of the lung. It is seen here on both volume and pressure tracings. If in such a state the lung be distended rather more forcibly for two or three inflations, and then the respiration be continued exactly the same as before, it will return with very considerable accuracy to its original state. The lung therefore behaves in this respect in exactly the same way as imperfectly elastic bodies, showing a higher degree of extension if it be brought down from a high tension to a moderate one, and a lower degree of extension if brought up from a lower to the same moderate tension.

The lower limiting line at first falls and only later rises. In spite of this fall the two limiting lines approach one another as we have already seen, and it is the approximation of the two lines—*i.e.*, the diminution in amplitude—which is the sign of bronchial constriction and not the change in direction of the two lines. This fall in the lower line is due to a decrease in blood-volume, a change which is discussed on p. 134.

We may now pass on to an examination of the similar effects observed in the rabbit. A typical tracing is given in Fig. 11, which we see is practically the same result as that just discussed, though the commencement of the change is a little different. This is seen from the measurements of the heights of the air-waves. Before stimulation these are 33 mm., while those at the commencement of excitation are 36, 32, 29, 22, 19, 16, 15, and 14 mm. At this last point the minimum is reached. The important difference is that the first effect is an increase in height, which is accompanied by a sharp fall in the lower limiting line, a fall which is of vascular origin. Either, therefore, there is a preliminary transient bronchial dilatation, or the lobe is more distensible. That the latter is the true explanation was proved, for this animal, by stimulating the vagus immediately after the animal had been bled to death, when the preliminary increase was absent. The result therefore suggests that the empty condition of the pulmonary capillaries renders the lobe more distensible, a conclusion which we later show to be the correct one (*vide* p. 135). The only other notable difference between this and the preceding Figure is the rapid and more complete recovery.

In the third animal, the cat, the results obtained differ materially from those just described in the dog and rabbit, as is seen from Fig. 12. Here again the three tracings represent tracheal pressure, lung volume and aortic pressure. During the time indicated by the signal the right vagus was excited with weak induction shocks. The first effect is a slight diminution in amplitude, immediately followed by recovery to exactly the same height as before, and this continues during the whole duration of the stimulus; but 10 seconds after it had ceased a marked constriction commenced, attained its maximum 10 seconds later, and then gradually disappeared. The tracheal pressure shows analogous changes, though the artificial respiration was not arranged for showing them in a pronounced manner. The final relaxation is particularly slow in this instance, but commonly it is more complete and rather quicker.

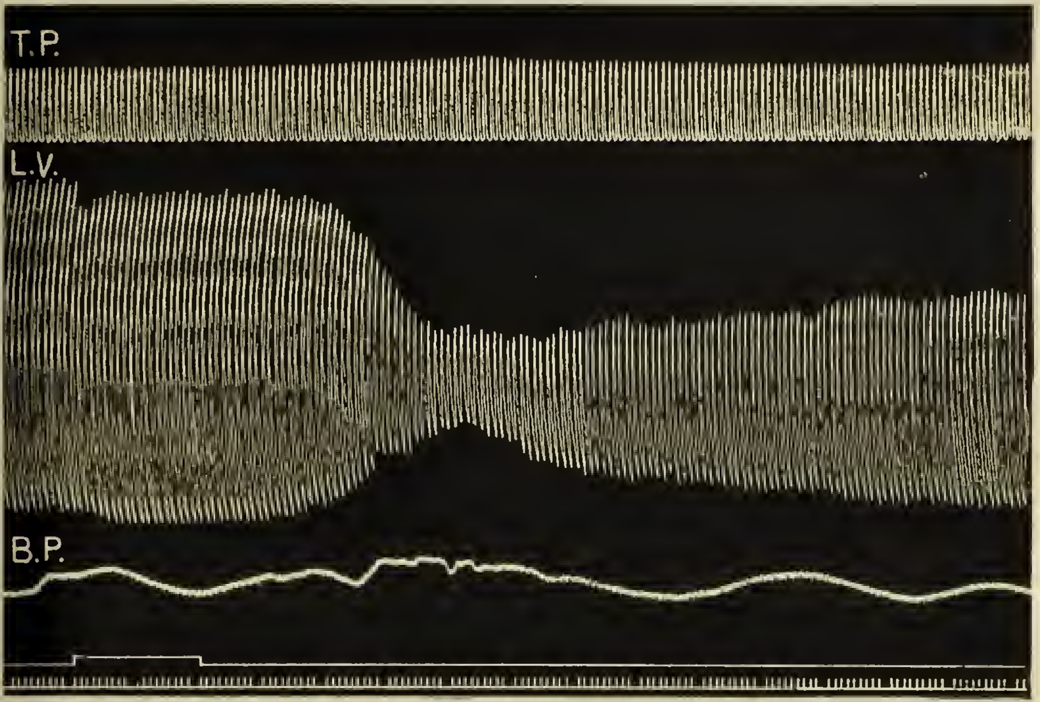
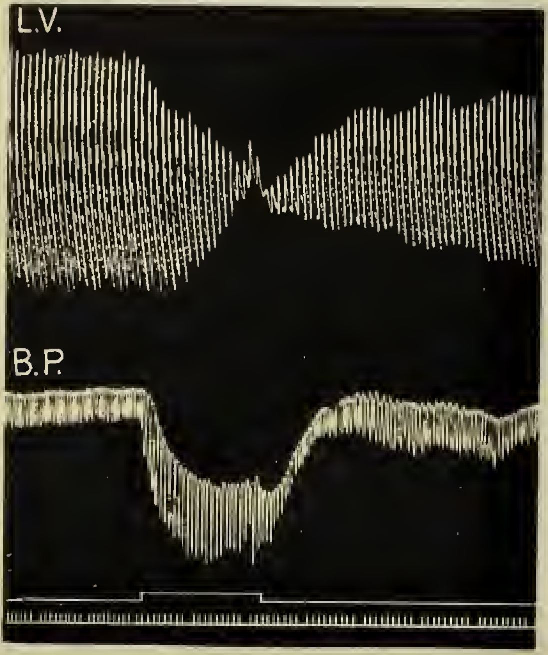


Fig. 12. Cat killed by pithing. Stimulation of vagus; coil at 10 cms. Zero B.P. 17 mm. below abscissa line.

Such a response as that figured at once suggests that we are here dealing with two simultaneous effects, the one tending to produce constriction, the other relaxation, *i.e.* that in the vagus there are broncho-dilator fibres as well as broncho-constrictor. The different response in the cat would then imply that the dilator fibres are especially developed in this animal. Although this figure shows the usual reaction obtained when experimenting upon the cat, yet we have observed many variations. A very rare effect is that recorded in Fig. 13, which we see differs in no essential particular from the results typically obtained in the dog and rabbit. The constriction had become practically complete when the excitation was stopped. It is interesting too to note that in this case the cardiac inhibition is much more pronounced than is usual in this animal. We have at different times observed practically all intermediate stages between these two extremes. Thus, the initial constriction may be more prolonged, but the chief variations were found in the constriction which follows the cessation of the stimulus. This may commence a little earlier and be more marked, but the effect is generally in the reverse direction. As examples of the more commonly observed variations we give the following four figures. In the first of these (Fig. 14) a rapid constriction is produced, which quickly recovers although the stimulation continues, and there is no constriction after the cessation of the stimulus. In Fig. 15 the first effect of the prolonged stimulus is a relaxation, followed by a constriction, but again, the main constrictor effect is observed after cessation of the stimulus. This experiment is of further interest because the animal had, at some time, suffered from a severe attack of pleurisy on the right side and the lobe experimented upon was firmly adherent to the outer pleura, so that considerable difficulty was experienced in separating it



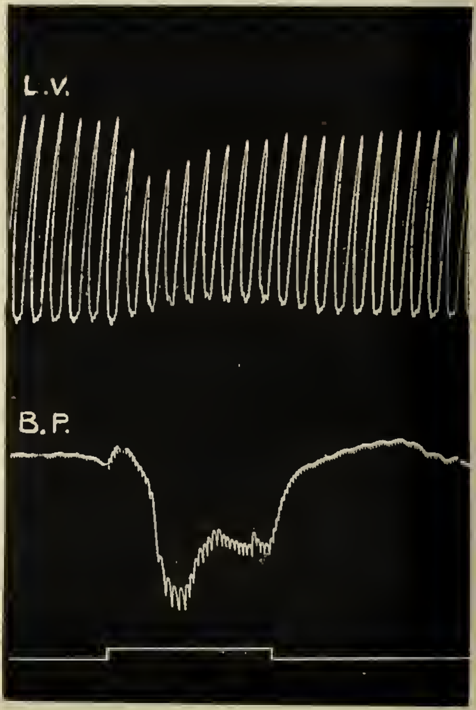


Fig. 13. Cat. Pithed. Stimulation of vagus ; coil at 6 cms. Zero B.P. 24 mm. below abscissa.

Fig. 14. Cat. Ether. Stimulation of vagus ; coil at 10 cms. Zero B.P. 48 mm. below abscissa line. Reduced $\frac{1}{3}$.

from the chest wall. The lobe was markedly emphysematous and it is seen from the tracing that retraction was very imperfect. Two further tracings taken from this same animal are given as they illustrate other points. In Fig. 16 very considerable distension is produced, which begins about 5 seconds after the stimulus was started. In this experiment the pressure of distension was kept very low and the time allowed for expiration was shortened. The tracing of Fig. 17 was obtained from the same animal about 1 minute later. The force of inflation had been slightly increased, but the time allowed for expiration remained the same as before. At *a*, the artificial respiration was stopped for a short time, in order to record the amount of residual distension of the lobe, which is seen to be very considerable. The vagus was now excited, and at once the amplitude falls and further over-distension is produced. It is interesting to note that the instant at which excitation ceased is marked on the volume record by a sudden diminution in amplitude of the air-waves, which shortly after are cut out completely. About 2 minutes later the air movements returned spontaneously. The experiment therefore has an important bearing upon the pathology of asthma, for it is one in which we were able to observe the changes in the air movements when the bronchioles

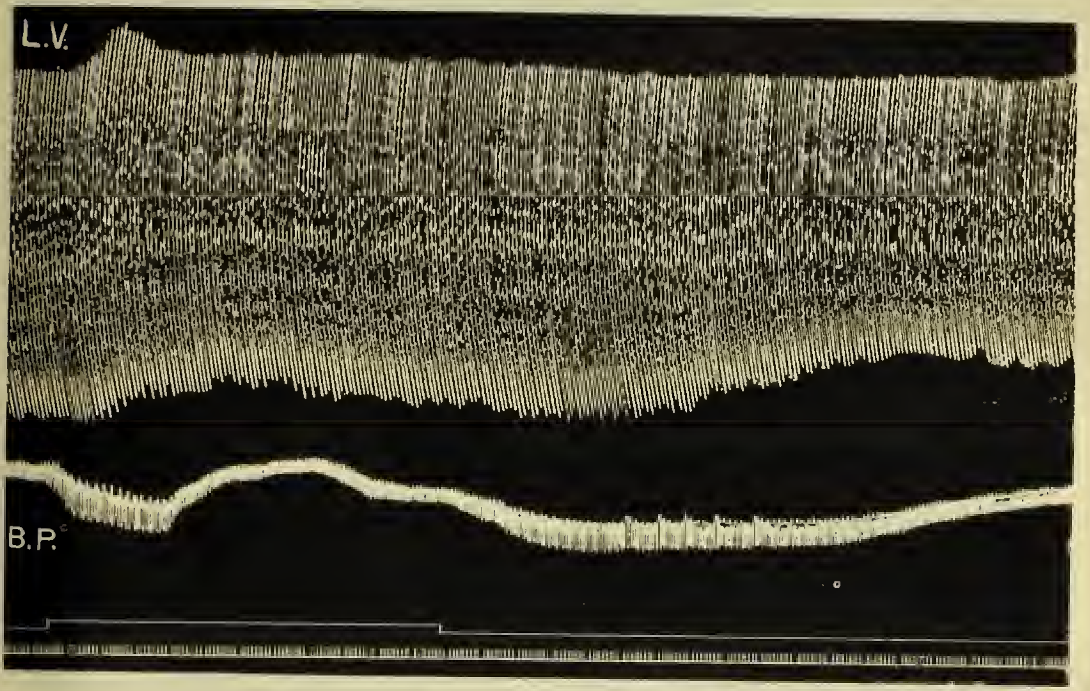


Fig. 15. Cat. A.C.E. Stimulation of right vagus ; coil at 8 cms. Reduced ½.

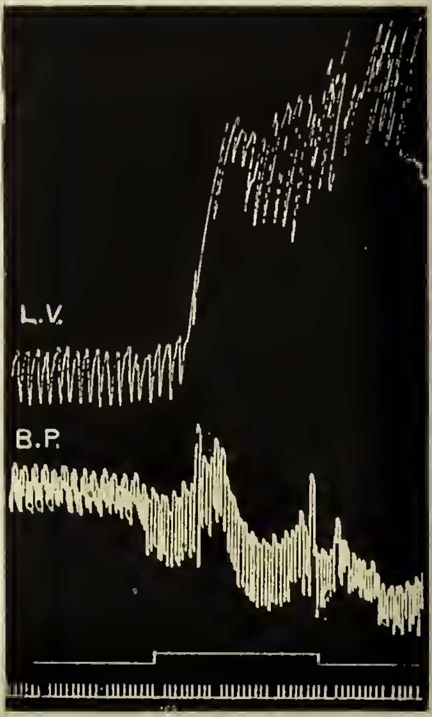


Fig. 16. Cat. A.C.E. Respiration effected by the method described on p. 148. Stimulation of vagus; coil at 8 cms. Pressure of inflation 38 mm. of water. Zero B.P. 38 mm. below abscissa line. Reduced $\frac{1}{3}$.

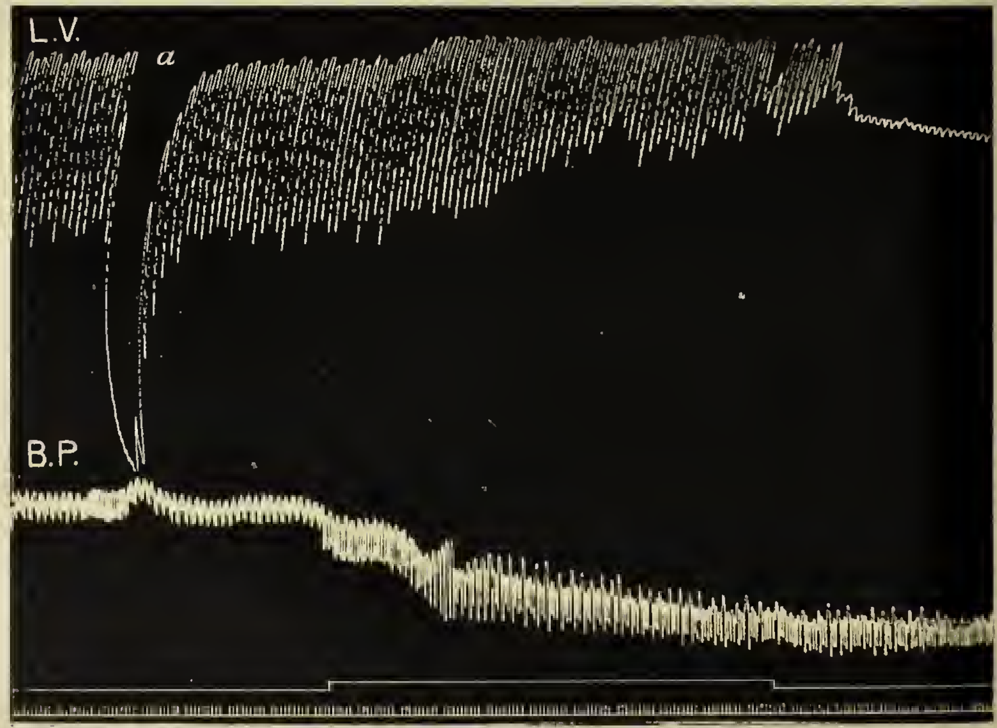


Fig. 17. Same animal as in Fig. 16. The artificial respiration effected by a rather greater pressure of air (50 mm. of water). Zero B.P. 20 mm. below abscissa line. × $\frac{1}{2}$.

constricted in an emphysematous lobe. Before enclosing the lobe in the oncometer it was watched and noted to expand very easily but to retract slowly and incompletely. In appearance the lobe was much paler than the upper part of the lung, which was apparently normal. The alveoli were very large. The tracing exemplifies the abnormal physical state of the lobe very completely. Its distensibility was if anything rather increased but its elasticity was very imperfect. Lastly, the time required by the lobe to empty itself was much greater than in the case of a normal one.

B. *Experiments upon partially collapsed lobes.*

Experiments upon partially collapsed lobes show certain differences from those already described. Fig. 18 gives one of the commonest results we have observed in the cat, and it resembles in the main the usual result obtained in this animal, shown in Fig. 12. A short constriction marks the commencement of excitation; but the air movements are unaltered during the main period of excitation, and the main constriction developes during the 70 seconds after the stimulation was stopped. It results in almost complete blockage of the air movements. The alterations in the lower limiting line seen in this figure are usually present and though of a degree which might readily be caused by blood-volume changes, we do not think this is the true explanation here, for the aortic pressure remains practically unaltered. They are air-volume changes due, probably, to changes in the distensibility of the alveoli caused by variations in the amount of blood in

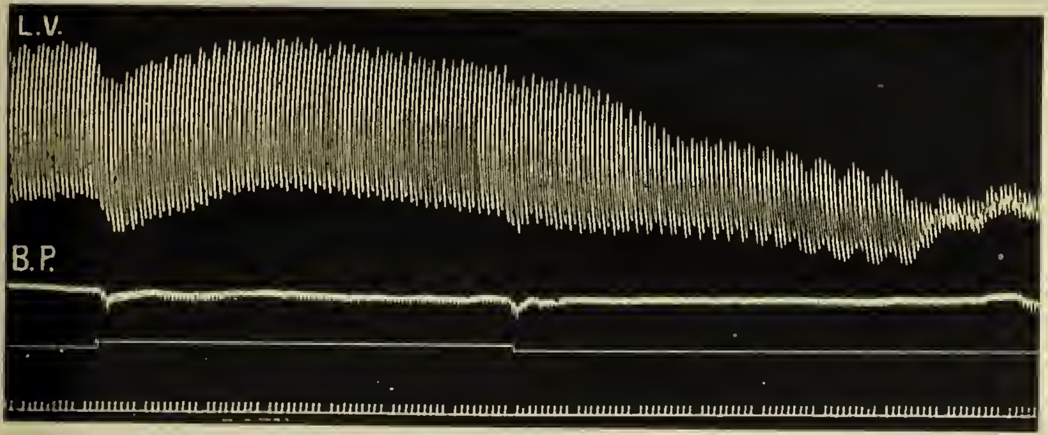


Fig. 18. Cat. A.C.E. Stimulation of vagus; coil at 7 cms. Zero B.P. 73 mm. below abscissa line. Reduced ½.

the pulmonary capillaries. Just before the air-waves are cut out, a few marked waves are seen in the limiting lines. These are not interference waves, for their amplitude is too great in comparison to the volume-pulse, nor does the crest on the one line correspond to the trough on the other. For this latter reason they cannot be tonus waves and must therefore be variations in the quantity of air retained at the end of expiration, due apparently to slight changes in the ease with which the lobe empties itself in expiration, for the fall of the line as the waves descend on the limiting lines is greater than in the ascending portions. The explanation of this variation in rate of emptying is not easy to understand, and a further instance of it is seen in the over-distension produced when the constriction finally cuts out the air-waves. They may possibly be due to variations in inflation of different sets of alveoli. A lobe which has completely collapsed in this way usually remains so, although the bronchial constriction may have passed off. A few inflations, of sufficient force to slightly distend it, are enough to cause a return to the original state if the bronchial constriction has passed off, but if this has not disappeared they simply produce distension. As in the previous case (Fig. 12) the absence of constriction during excitation is to be explained by the simultaneous excitation of the broncho-dilators.

In Figs. 19 and 20 various modifications of the results obtained in this group of experiments are given. Fig. 19 is an instance showing slow onset, mainly occurring after excitation had ceased, and followed by spontaneous recovery. In Fig. 20 one other condition is illustrated,

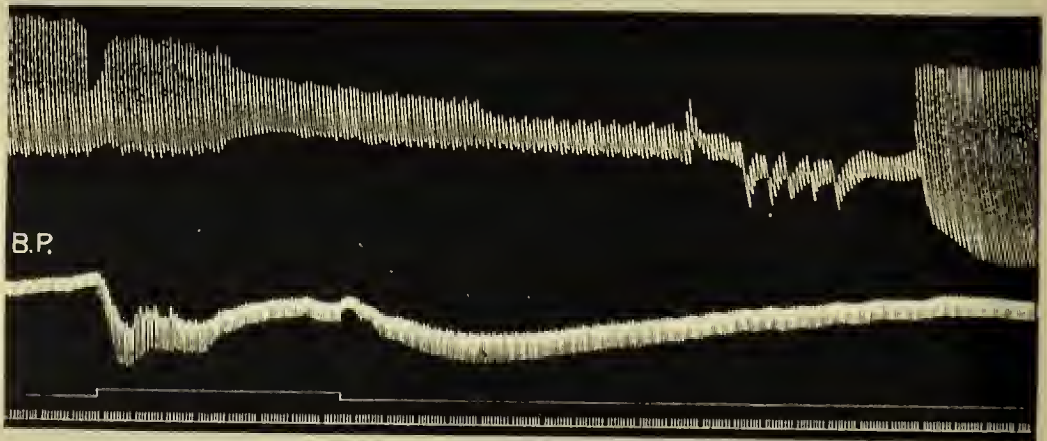


Fig. 19. Cat. Ether. Stimulation of vagus; coil at 8 cms.
Zero B.P. 15 mm. below abscissa line. Reduced ½.

viz. distension, and the course of the constriction is very irregular and in waves. As these occur the lobe becomes more and more distended, the alveoli experiencing considerable difficulty in emptying themselves. The condition lasts a long time after the excitation has ceased, and finally as the distension gradually falls spontaneous recovery occurs. The question as to whether or no distension is produced is largely determined by the way in which artificial respiration is effected. If this is rapid and produces fair dilatation of the lobe while the time allowed for expiration is short, distension is commonly produced. Up to a certain point the greater the contraction of the bronchioles the more readily it occurs, but if the constriction is very marked, quick in onset and prevents the entrance of air almost completely, it is not produced.

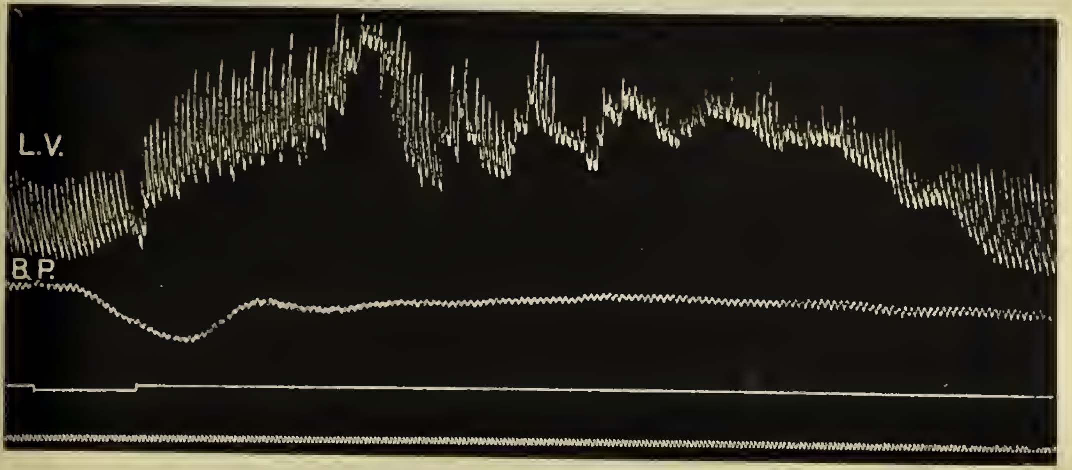


Fig. 20. Cat. Urethane. Curare. Stimulation of right vagus; coil at 7 cms. Zero B.P. 24 mm. below abscissa line. Reduced $\frac{1}{2}$.

C. *The negative results and their cause.*

In our earlier experiments we always worked upon partially collapsed lobes, and in these we usually obtained positive results following excitation of the vagus. When later we began to record the full air volume changes of the lobe, we were surprised to find that the excitation now usually gave poor and transitory effects, and that after the first excitation commonly no results whatever were obtained. Working under exactly the same conditions we at times obtained quite typical results. We never obtained completely negative results on the first excitation, but effects such as that shown in Fig. 21 were extremely common. It seemed to us that the cause might lie in one or more of the following, viz., (1) that the method was not sufficiently sensitive; (2) that there were broncho-dilator fibres present with the constrictor fibres in the vagus; (3) that faradic stimulation was not the best for the purpose; (4) that the cat, the animal which usually gave the negative results, only possessed few constrictor fibres in the vagus; (5) that it was due to the anæsthetic employed. We may at once dismiss the first of these, for we now have plenty of evidence that the method is as sensitive as could be desired. With regard to the second we were soon able to demonstrate the existence of broncho-dilator fibres, and for a long time thought this was the full explanation of our negative results. Our experiments upon the third possibility tended to confirm us in this, for having noted how frequently slight positive effects were obtained at the commencement and end of excitation, we tried the effect of repeated short periods of excitation with short intervals of rest. In a great number of instances this was found to be much more effective, but still the number of negative results was very unsatisfactory. We eliminated the possibility of their being due to local fatigue or to the local action of the anæsthetic, as a freshly exposed piece of nerve, thoroughly protected from any possibility of injury, gave the same negative results on excitation. We experimented with mechanical, thermal, and chemical stimuli, again with negative results. We also employed induction shocks in which the make and break shocks were equalised. The Helmholtz and Bernstein modifications gave no different effects, as was also the case when we used the

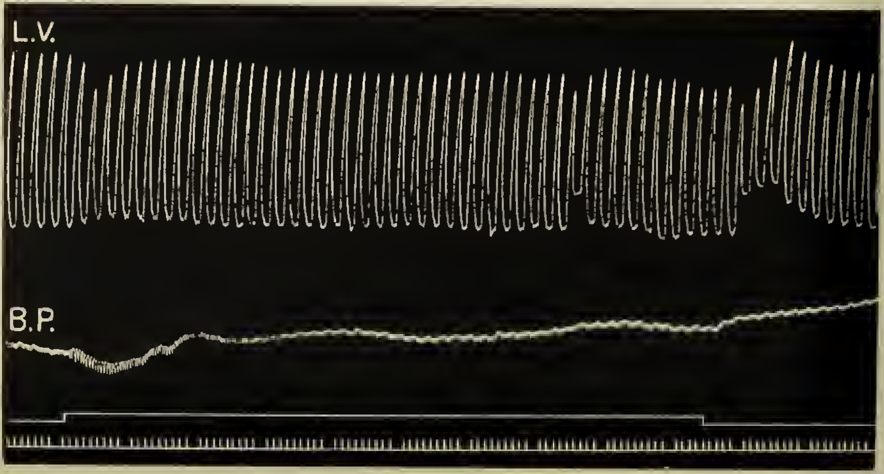


Fig. 21. Cat. A.C.E. Stimulation of right vagus; coil at 5 cms. Zero B.P. 21 mm. below abscissa line. Reduced ½.

house-current working through an incandescent lamp, which as has been pointed out by Locke[1] equalises the make and break shocks very efficiently. We tried further the effect of stimulating by single induction shocks repeated at one second and at two seconds interval, but again with negative results. The action of constant currents applied in various ways was equally ineffective. We therefore concluded that excitation in the ordinary manner was on the whole the best. The fourth suggestion mentioned above is important. Negative results were far commoner with the cat than with either the dog or the rabbit, a result which can be readily understood from a comparison of the three typical figures already given (Figs. 10, 11, and 12).

Unfortunately most of our work was completed before we directly tested the influence of the anæsthetic. To avoid all use of anæsthetic the animal was pithed, the medulla and brain only being destroyed. A tracheal cannula was then inserted and artificial respiration started. The operation was then carried out as usual. In these experiments we always obtained good positive effects, no matter how frequently the vagus was excited. In some experiments the nerve was stimulated as many as thirty times and with as good a result at the last excitation as at the first. It is from experiments of this kind that we are able to state that the usual type of reaction yielded by the cat's lung differs from that given by the rabbit or dog. The action of the anæsthetic is a peripheral one and probably at the nerve terminal in the bronchioles. Here the fibrils are exposed to the direct action of the volatile anæsthetic, and they are quickly affected by it. Still it is difficult to understand why the first excitation should cause, as it does, so much better contraction than any of the succeeding ones, although in many experiments we allowed ample time for recovery to take place. We think that the explanation of the negative effects being so much less common when experimenting upon partially collapsed lobes, is that in these the movement of air through the bronchioles was much less, the supply of anæsthetic was thus less frequently renewed, and the nerve endings consequently less exposed to its action.

We may here state that we have tested the branches leaving the ganglion stellatum for broncho-motor fibres with completely negative results.

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[1] Locke. *Proc. Physiol. Soc.* p. xvii. This *Journal*, XXVIII. 1902.

iii. Some other effects occurring with the excitation of the vagus.

As already pointed out, the first stage of the vagus effect is often, especially in the rabbit, accompanied by a marked fall in the lower limiting line, which as we have seen is caused by vascular changes simultaneously induced by the excitation. In the present paper we do not propose to discuss fully the effects upon the air movements caused by vascular changes, and in what follows only refer to changes of such magnitude that they might interfere with the proper interpretation to be placed upon the recorded effects.

An instance of such a case has already been given in Fig. 11, p. 123, and a further example is given at A, Fig. 22. The second half of this figure (B) was obtained in the following manner. After recovery from the first excitation the artificial respiration was stopped for a time, when the lobe collapsed considerably, the vagus was then excited a second time (at *a*). A second sharp fall in the tracing occurs, which is therefore due to a diminution in the blood content of the lobe, and there is a partial recovery as the inhibition passes off, and the blood-

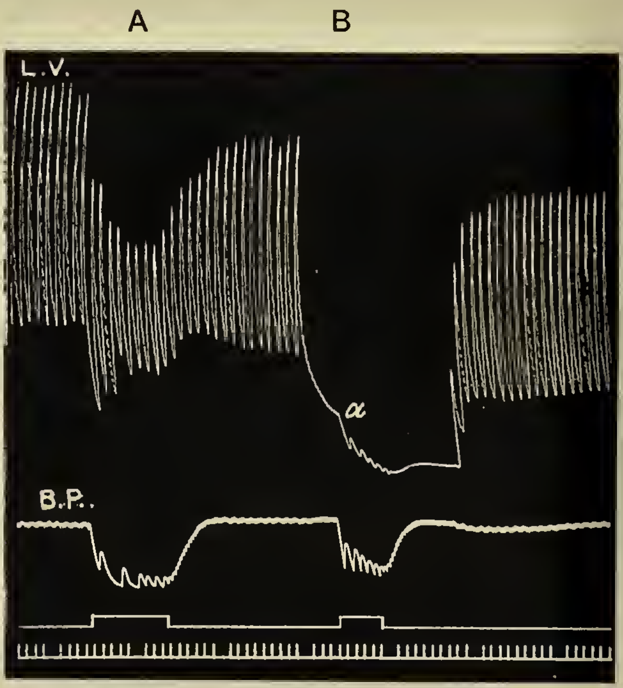

Fig. 22. Rabbit, pithed. Stimulation of vagus; coil at 10 cms. The first stimulation is during the artificial respiration and the second a short time after it had been stopped. Reduced ⅓.

pressure once more rises. Though the marked fall seen in the first of these two reactions is therefore partly a blood-volume diminution, the second reaction clearly indicates that this explanation only accounts for a part of the effect. That the whole effect is in fact due to a change in the blood distribution was proved in the following way upon the same animal. A control excitation was first recorded and the animal was then bled to death. When the blood ceased to flow the ventricles were cut across to make certain that no alteration in the amount of blood in the lobe was possible. The vagus was now excited and the reaction given in Fig. 23 was recorded. The result is as typical, as regards the air changes, as before, but differs from the preceding effects in that the lower limiting line begins to rise at once. We have another proof in the behaviour of the lobe in experiments in which the cardio-inhibitory fibres have degenerated. We found that the broncho-constrictor fibres were still excitable and the reaction was similar to one obtained upon the bloodless lung. Similarly too the initial fall in volume is absent if the vagus be excited while the vessels to the lobe are temporarily ligatured. Attempts to exclude the cardio-inhibitory influence by administration of atropine proved as ineffective in our hands as in Einthoven's[1] or Beer's[2], for we invariably found that the minimum dose of atropine sufficient to cut out the vagal action on the heart also abolished every effect upon the bronchial muscles. Beer[3] overcame the difficulty of separating the cardio-inhibitory effects from the broncho-constrictor by the employment of muscarine. As is well known[4] a stage in muscarine poisoning occurs in which cardiac inhibition is present, but no further slowing occurs on exciting the vagus. Working upon animals in which this condition was present, Beer states that he was able to obtain typical broncho-constrictor effects without a simultaneous vascular change as evidenced by absence of change in the aortic blood-pressure.

The effect we are dealing with is therefore due to the altered distribution of the blood, but Fig. 22 tends to show that the full volume change is a decrease partly in blood, partly in air. How then are we to explain the air diminution as consequent to a blood change? As it is due to a decrease in the amount of blood in the lobe, the explanation probably is that the empty state of the pulmonary capillaries enables the alveoli to contract more completely. The erectile action of the capillaries has diminished, and we are in fact dealing with a change in the condition which v. Basch[1] has termed 'Lungenstarrheit.' This change in distensibility[2] together with a diminution in the blood-volume of the lobe therefore explains the sudden decrease in volume of the lobe occurring at the commencement of excitation of the vagus. It is not always present and is usually far better marked in the rabbit than in either the dog or the cat. In the latter it is commonly absent or but very slightly marked[3], a result which agrees with the commonly observed slight action of the vagus on the heart. The effect is, as was to be expected, more marked the higher the ratio of the volume-pulse to the air volume. It is of considerable

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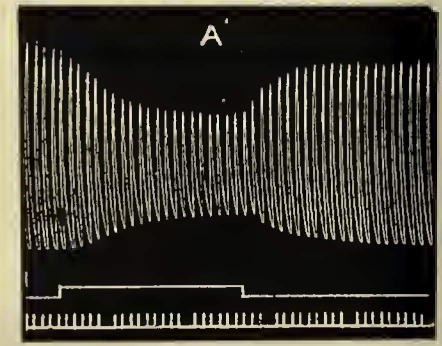
[1] Einthoven. *loc. cit.* p. 428.

[2] Beer. *loc. cit.* p. 123.

[3] Beer. *loc. cit.* p. 150.

[4] Weinzweig. *Arch. f. (Anat. u.) Physiol.* 1882, p. 527.



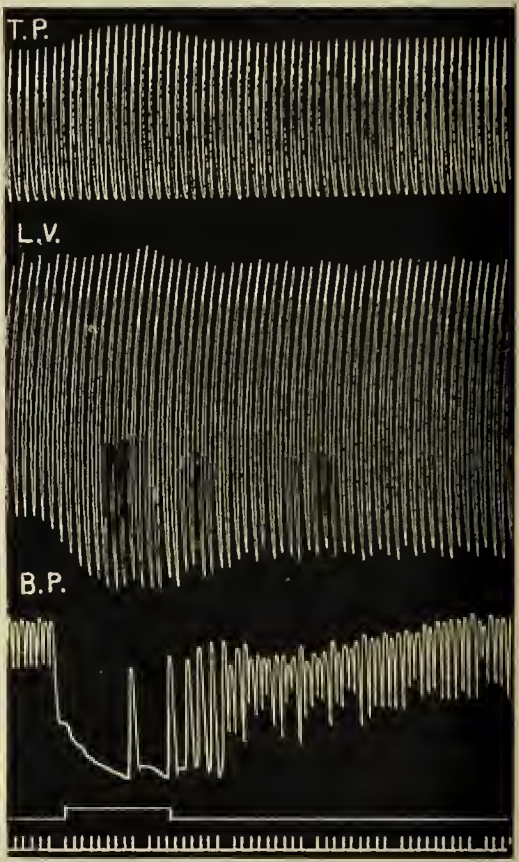


Fig. 23. Same animal as in Fig. 22 rendered bloodless by opening the carotid and excising the heart. Stimulation of vagus; coil at 10 cms. Reduced ⅓.

Fig. 24. Dog, pithed. Stimulation of left (opposite) vagus; coil at 8 cms.

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[1] v. Basch. *Wien. med. Blätt.* 1887, p. 465, also *Wien. med. Presse.* XXIX. p. 587. 1888.

[2] The question of distensibility will be discussed by us more fully in a later paper.

[3] See, however, Fig. 18.

value to note that stimulation of the vagus leads to a fall in blood content of the lung. This means either that the output from the left ventricle is greater than that from the right, and therefore that the left ventricle is less inhibited than the right, or that the lung empties itself into the left auricle, which thus becomes over-distended. The reaction is the same on stimulation of either the right or left vagus. Compare in this connection Fig. 24, which records the effect on the right lung of excitation of the left vagus.

Another point, arising from the effect of excitation of the vagus, is the influence of the rise of tracheal pressure, produced in some instances by our method of experimenting, upon the remaining lobes of the lung. It appeared possible that the lobe experimented upon, being placed under very dissimilar conditions from the rest of the lungs, might react differently, and that therefore many of the negative results we have previously described and explained might have been due to this cause. A bronchial constriction may cause a rise in tracheal pressure under the conditions of our experiments, and this rise will tend to diminish the effect upon the amplitude of the air-waves. It must also produce a greater distension of those lobes not involved in the bronchial constriction, which is in fact seen to be the case in Fig. 24. This tracing gives a record of the tracheal pressure, volume changes of a lobe of the right lung, and the aortic pressure curve in a dog. At the time indicated by the signal the left (opposite) vagus was excited. This caused a constriction of the bronchioles of the left lung as indicated by the rise in tracheal pressure. This rise in pressure caused an increase in the amplitude of the air-waves of the record of the right lobe. The maximum tracheal pressure per inflation rose from 144 mm. before to a maximum of 171 mm. of water during the excitation. The increase in the amplitude of the air-waves is from 41 mm. to a maximum of 54 mm. The rise in tracheal pressure and the air volume changes both occur gradually and the maxima coincide in point of time. The increase in amplitude of the air-waves is however proportionately greater than the increase in pressure. Within certain limits we have seen that the amount of distension of the lobe fairly accurately follows the increase in tracheal pressure, in direct proportion. If this were the case in this experiment the amplitude of the air-waves should only have increased to 48·5 mm., whereas actually they increased to 54 mm. The explanation of this further increase is that, as already noted, the extensibility of the lobe is greater because the cardiac inhibition has partially emptied its capillaries. In this figure we may also draw attention to the fact that excitation of the left vagus has produced a fall in volume of the lung.

This experiment is also of service as tending to show that no constrictor fibres for the right lung ran in the left vagus. It is however possible that some of the increase of distension of the right lung might have been due to the excitation of dilator fibres for the right lung in the left vagus. This is not probable, for we have much evidence to show that in a resting state the bronchioles are completely relaxed. We have, moreover, proved that no crossing of the fibres occurs in the thorax, by first plugging the left bronchus with vaselined cotton-wool and then exciting the left vagus. The blood change alone, *i.e.* decrease in volume and proportionate increase in distensibility, is now produced in the right lung.

iv. Does the vagus exert a tonic effect upon the bronchial muscles?

We have already mentioned (p. 120) the observations of Chauveau and of Roy and Brown on this point. Einthoven[1] was unable to observe any dilatation of the bronchioles on section of the vagi in the dog, unless some tone had been accidentally or artificially induced at some previous time in the experiment. In the dog, cat, and rabbit we have never observed dilatation nor constriction on section of the vagus at the commencement of an experiment. We have however recorded dilatation following section in animals which have been previously injected with small doses of muscarine or pilocarpine. The dilatation in these rare cases has never been very great and could quite well be explained as due to excitation of the dilator fibres by the section of the nerve. We have also observed dilatation in animals which have been previously receiving large doses of carbonic acid. This dilatation is often very marked, and as will be shown later (see p. 167) is due to the cutting off of impulses from the centre in the medulla.

SECTION 6. BRONCHO-DILATOR NERVE-FIBRES.

Whether dilator fibres are supplied to the bronchial muscles has been studied by many previous workers. Thus Sandmann[2] comes to the conclusion that such fibres are present in the vagus, but the figures he gives are far from conclusive, especially as he has not properly eliminated the effects of vascular changes. Roy and Brown[1] describe dilator fibres as being present with the constrictor in the vagus. They rely mainly upon reflex dilatation of the bronchioles, but give no evidence to show how they eliminated the possibility, that the result was due to an inhibition of the tonus maintained by the medullary centre, for in their method of experimenting tonus is always present. It must be remembered too, that their experiments refer to bronchi of some considerable size. Einthoven[2] comes definitely to the conclusion, that there is no evidence in favour of the existence of dilator fibres which cannot be better explained upon other grounds. In this conclusion he is confirmed by Beer[3], who however in an addendum[4] to his paper states that in two experiments upon animals previously treated with muscarine he observed increase in the amplitude of the phrenographic curve following excitation of the vagus. His records show a small but definite increase which gradually dies away after stimulation ceases. He suggests that possibly this may mean a broncho-dilatation, although he does not commit himself so far as to regard the result as evidence of the existence of broncho-dilators.

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[1] Einthoven. *loc. cit.* p. 411.

[2] Sandmann. *loc. cit.*

Our attention was drawn early to the possibility of the presence of dilator fibres in the vagus by the irregularity of the reaction of the bronchioles, when the vagus was excited in the cat. It was useless attempting to prove their existence by trying to find a form of stimulus that would leave the constrictor fibres unexcited, because the bronchial muscles when at rest show no tonus. In our first attempts we therefore threw the muscles into tonic contraction and then excited the vagus in the ordinary manner. We induced the tonic contraction of the bronchioles by injections of muscarine or of pilocarpine, and at once obtained positive evidence of the presence of dilator fibres, as will be seen from Fig. 25. Excitation of the vagus is here seen to produce a big effect upon the volume record of the lobe, which begins to increase in amplitude after a short latency, quickly attains a maximum at which it persists while the stimulation lasts, and dies away after its cessation. A reaction of such magnitude as this can only be due to the one cause, namely, a relaxation of the bronchial muscles. This conclusion is strengthened by the record of the tracheal pressure, the maximum height of which per inflation is seen to fall as the lung record increases and to rise as the lung again diminishes. It is most striking to watch the quick recovery of the whole lung which previously may have been almost quiescent. The reaction may be repeated an indefinite number of times. The fibres are not easily fatigued and the lung may be kept relaxed for a considerable time by continuing the excitation of the nerve, but as soon as the stimulation is stopped the dilatation quickly passes off, and the constriction gradually returns. The behaviour of these fibres shows many points of similarity with that of the constrictor fibres, more particularly in regard to their sensitiveness to anæsthetics administered by inhalation. Quite a small dose of the latter markedly diminishes the effect.

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[1] Roy and Brown. *loc. cit.* p. xxiii.

[2] Einthoven. *loc. cit.* p. 417.

[3] Beer. *loc. cit.* p. 133.

[4] Beer. *loc. cit.* p. 208.

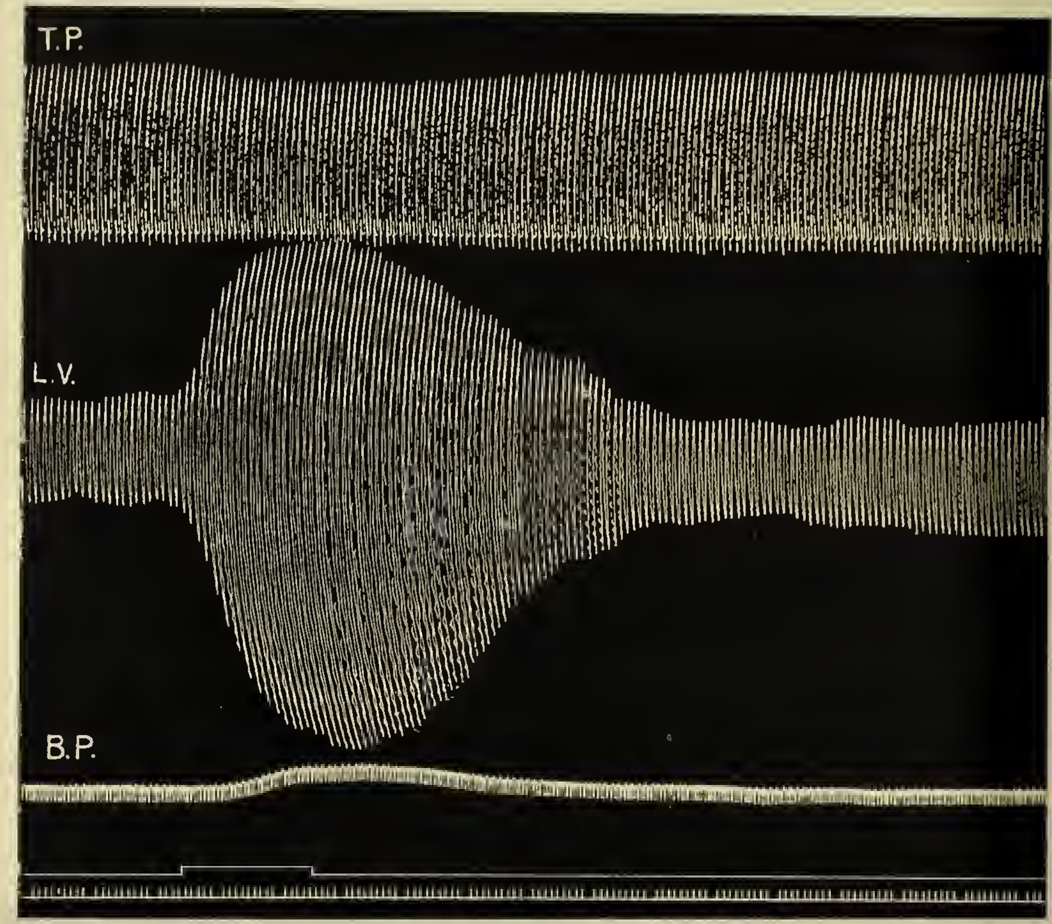

Fig. 25. Cat, pithed. Excitation of right vagus, coil at 4 cms. during the bronchial tonus produced by 0·01 grm. pilocarpine.

We have attempted to separate the constrictor from the dilator fibres by the degeneration method. The vagus was cut in the neck and the peripheral end excited 4, 7, 14, and 56 days after section. Stimulation of the vagus 4 days after section gave a typical reaction similar to that of Fig. 12. Pilocarpine caused constriction in the usual way and good dilator effects were then obtained. The animal in which the nerve had been cut 7 days previously gave less marked results. The constrictor effect was distinctly weaker but the muscles reacted to pilocarpine well and the subsequent dilator effects were nearly as good as those obtained from a normal nerve. In the animal in which the nerve had been cut 14 days the changes were more pronounced. The constrictor effect on vagal excitation was very weak, even though interrupted excitations were employed. Pilocarpine still caused a fairly typical constriction and the dilator fibres were apparently well preserved, being still nearly as good as in a normal animal. The animal whose vagus had been cut 56 days previously gave most interesting results. We could not obtain constriction by any mode of excitation of the nerve. Pilocarpine produced absolutely no constriction of the lung on the same side, although it produced the typical effect upon the opposite (intact) side. Excitation of the nerve after the administration was also without effect. We therefore conclude from these degeneration experiments that the constrictor fibres degenerate somewhat earlier than the dilator, and that both take a considerable time to completely degenerate. As pilocarpine produced no effect upon the last animal we further conclude that the drug acts upon the nerve terminals of the constrictor fibres. Our results upon the degeneration of the broncho-constrictor fibres are thus somewhat different from those obtained by Bert[1], who found them degenerated in the same time as the cardio-inhibitory fibres, *i.e.* in 4 to 5 days. He found too that the pulmonary contractility had entirely gone in 2 weeks and that it had not returned after 4 months.

In considering whether our experiments really prove the existence of dilator fibres in the vagus, we have to eliminate the possibility that the effect might be due to an inhibition of the stimulating effect of the pilocarpine upon the constrictor nerve terminals and not to an action of a separate set of nerve-fibres upon the muscles themselves. Also it is well known that there are many ganglionic masses scattered along the bronchi and it is therefore possible that the inhibition may occur here. Our degeneration experiments, however, show that the constrictor fibres do not connect with these ganglia. We think, moreover, that the magnitude of the effect, and the regularity of its course in successive stimulations, are points which prove the existence of dilator fibres. This conclusion is greatly strengthened by the behaviour of the animals in which the vagi had been cut some time previously, for in these, as already stated, we succeeded in partially separating the two sets of fibres. Again, the form of reaction which is typically presented by the cat is one which is difficult to interpret except on the supposition that we are here dealing with the simultaneous excitation of two opposing sets of nerve-fibres. The question is one to which we hope to return at some future period.

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[1] Bert. *loc. cit.* p. 370.

In connection with the question of broncho-dilator fibres some of Grossmann's[1] experiments with muscarine are of importance. He states that the restriction of the air movements in animals treated with muscarine, which in his first paper he entirely ascribed to vascular changes, may be annulled by stimulating the cardiac accelerators. In the tracing Grossmann reproduces (Fig. 7, Pl. vi.) the pressure changes in the aorta and pulmonary artery are very slight and had already commenced before the excitation was started, but the effect upon the respiratory movements was most marked. Before we read his paper we had tested the nerves leaving the ganglion stellatum to see whether they contained broncho-constrictor or dilator fibres with completely negative results. After reading his paper we again returned to those experiments, for we thought that the most probable explanation of his result was that some broncho-dilator fibres passed through the ganglion stellatum. Our results were again absolutely negative. We were not able to confirm his experiments in any of the few instances we repeated them.

We conclude that no dilator fibres to the bronchial muscles pass through the ganglion stellatum.

SECTION 7. REFLEX BRONCHO-MOTOR PHENOMENA.

Many previous observers have obtained evidence of bronchial constriction following various sensory stimulations. Thus Gerlach[2], by exciting the central end of the cut vagus or superior laryngeal, obtained a rise of tracheal pressure which was abolished by section of the second vagus: excitation of the recurrent laryngeal was without effect. Riegel and Edinger[3] showed that ammonia vapour blown into the lung caused constriction and that the effect was abolished by cutting the vagi. Roy and Brown[4] found that excitation of the central end of one vagus usually caused slight contraction, though at times and especially if the animal was etherised it caused a marked expansion. In these latter cases stimulation of the uncut vagus usually caused expansion. From excitation of the central end of a sensory nerve they very rarely observed any effect, and if any change occurred it was very slight. Stimulation of the stomach, intestine, pleura, tracheal mucous membrane or nostrils also proved ineffective. Franck[1] obtained positive results on exciting the central end of the vagus. The tracing of pleural pressure and of the respiratory movements, which he gives, only shows quite small changes, and he had not sufficiently excluded vascular alterations to enable us to definitely conclude that the effects were of bronchial origin. In a later paper[2] he describes the production of bronchial spasm following endo-cardial and endo-aortic irritation, but he gives no figures illustrating his experiments. Knoll[3], moreover, showed that the reflexes Franck observed were due to irritation of the pulmonary nerves and not of those arising from heart or aorta. Sandmann[4] obtained reflex constriction from irritation of the nose, larynx, and even of the cornea, but the curves he gives in support of this are not very convincing. Lazarus[5] obtained reflexes by stimulating the nasal mucous membrane mechanically or electrically; they were abolished on section of the vagi. He found that the most sensitive spots were the nasal septum and the turbinals.

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[1] Grossmann. *Ztschr. klin. Med.* XII. p. 579. 1887.

[2] Gerlach. *loc. cit.*

[3] Riegel and Edinger. *loc. cit.*

[4] Roy and Brown. *loc. cit.* p. xxiii.

The conclusions thus far enumerated are all based upon experiments in which the method employed was not very sensitive. Vascular changes may therefore have played a great part in their production and certainly had not been sufficiently eliminated. Einthoven's[6] method is much more trustworthy, and he describes the reflex results he was able to obtain as very inconstantly produced. Stimulation of the central end of the vagus gave nothing; of the sciatic usually nothing, but in one case he recorded marked constriction and in one other marked dilatation. In both these two exceptional cases the effect was cut out by dividing the two vagi. In the animal in which dilatation occurred he concluded that the result was not due to excitation of dilator fibres but to inhibition of a tonic action of the constrictor centre.

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[1] Franck. *Fonctions motrices du Cerveau*, p. 145, and *Arch. de Physiol.* XXI. p. 544. 1889.

[2] Franck. *Arch. de Physiol.* XXII. p. 546. 1890.

[3] Knoll. *Wien. Sitzungsber.* III. p. 479. 1884.

[4] Sandmann. *loc. cit.*

[5] Lazarus. *loc. cit.*

[6] Einthoven. *loc. cit.* pp. 413 and 419.

He found that this was the one animal, out of all he experimented upon, which showed considerable constrictor tone.

We have in many experiments attempted to obtain reflex constriction or dilatation of the bronchioles by stimulating afferent nerves, but while the direct excitation of the vagus gives very constant results reflex excitation is disappointingly irregular. The most important nerves we have stimulated for this purpose are the sciatic, the vagus, the laryngeal branches of the vagus, and the nasal branches of the fifth cranial nerve. In studying these reflexes we have found it of the utmost importance to avoid the use of chloroform or ether as the anæsthetic. The experiments must therefore either be performed upon unanæsthetised animals, upon animals anæsthetised with morphia, or upon decerebrate animals. Our experiments were usually conducted under one of the two latter conditions, but in a few instances were repeated upon animals lightly anæsthetised with chloroform.

In no instance have we obtained any effect by exciting the central end of the sciatic in a curarised animal, but the experiments are not numerous.

Stimulation of the central end of the vagus divided about the middle of the neck also produced no change, a result which confirms Einthoven's experiments of a similar character. In one instance however, we observed a slight but distinct dilatation (Fig. 26.) This result would seem to indicate the presence of some tonus in the bronchial muscles, a condition which may have existed here, for we had just previously excited the opposite vagus in its continuity in the neck. There is one other possible explanation of the result, which we think to be the more probable one. Although omitted from the tracing the excitation caused a marked fall in blood-pressure. This leads, as we have already seen, to an increase in distensibility of the alveoli by the emptying of the pulmonary capillaries and can well account for the

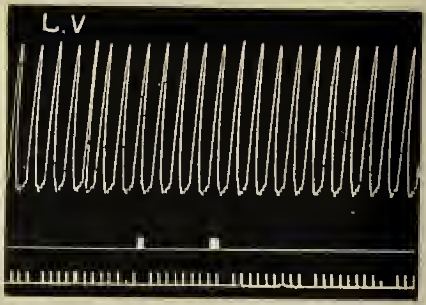

Fig. 26. Cat. A.C.E. Tie and cut right vagus.

small increase in amplitude of the tracing recorded. Excitation of the superior laryngeal has in the three instances we have tried it given no result.

The most important reflexes we have obtained are from the nasal mucous membrane. Excitation of this either electrically, mechanically, or chemically usually produces some degree of constriction, which, as a rule, comes on very gradually (Fig. 27) and is very persistent. In the instance given the stimulus was mechanical, but we found that in most cases chemical excitation gave the best results. A most effective stimulus for this purpose is bromine vapour blown in a diluted form over the nasal mucous membrane. It, however, has the great disadvantage that the stimulation cannot be repeated, for the first administration apparently kills the nerve terminals. In one instance only, this form of excitation produced an increase in the air-waves (Fig. 28). We have already pointed out that there is little or no tonus in these muscles and we are therefore inclined to attribute its presence in this instance to some accidental cause, though we were unable to discover its origin.

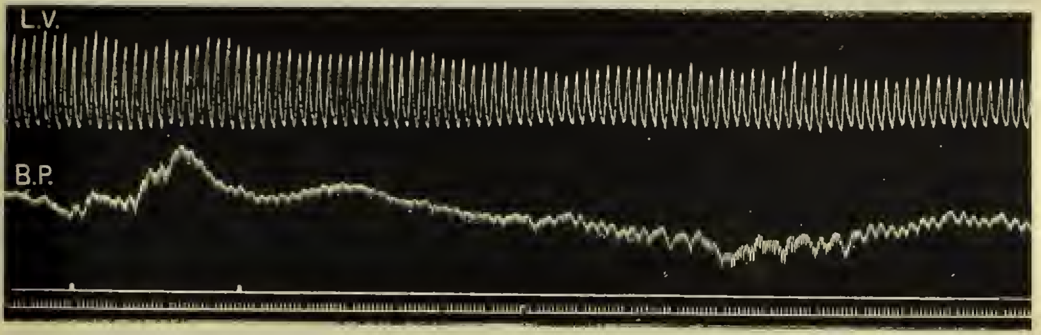

Fig. 27. Cat, decerebrate. Between the two signals the left nostril was mechanically excited. Zero B.P. 32 mm. below the abscissa line. Reduced nearly $\frac{2}{3}$.

Seeing that the existence of broncho-dilator fibres is now certain, there must be a double medullary centre, and it should be possible under appropriate conditions to set either of them into activity separately.

Broncho-reflex effects are of considerable importance as tending to elucidate certain points in the pathology of spasmodic asthma. We hope to return to them again later, but we may point out that those observers who have excited the nasal mucous membrane, all describe this as the most effective position from which to obtain reflex-bronchial constriction, and in this we agree. It has long been known that many cases of spasmodic asthma have been permanently cured by operations upon the nose. Recently Francis[1] has published remarkable results in which most cases of spasmodic asthma have been permanently cured by cauterisation of a certain area of the nasal septum. This area is a spot high up and towards the back. As confirming this we have found that the most effective spot for bringing about the reflex in cats and dogs is a small area well back upon the nasal septum. The reflex constriction from the nasal mucous membrane must be of a protective nature. We know that by excitation of this membrane the following reflex effects have been obtained[2]. (1) Closure of the glottis; (2) arrest or slowing of the respiration; (3) cardiac inhibition; (4) dilatation of the peripheral arterioles. Chemical or mechanical excitation of the laryngeal mucous membrane leads to arrest of the heart and of the respiration, and finally excitation of the pulmonary nerves leads to most pronounced cardiac inhibition. All these reflexes appear to be protective and for the purpose of saving the animal from the effects of

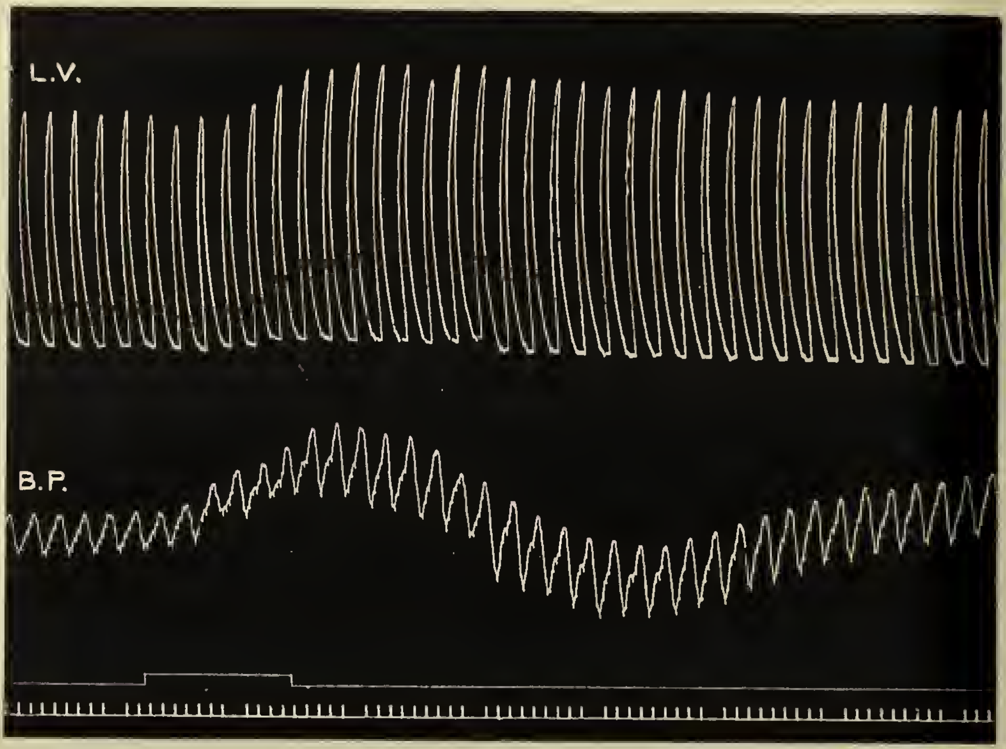

Fig. 28. Dog. Ether. Morphia. Bromine vapour blown into left nostril. Zero B.P. 30 mm. below abscissa line. Reduced $\frac{1}{3}$.

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[1] Francis. *Lancet*, Oct. 18th, 1902, p. 1053.

[2] See Brodie and Russell. This *Journal*, xxvi. p. 92, 1900, where an account of the earlier literature is given.

irritant particles or vapours entering with the air in respiration. All the reflexes upon the respiratory tract tend to stop the entrance of any irritant, effects which are all the more perfectly realised because the first part of the respiratory tract is so sensitive to these forms of excitation. A still further protection is reached by the vascular effects, for these in the first place tend to make the animal faint and thus reduce the activity of the respiratory centre, and secondly tend to retard the absorption of any irritant vapour that may have gained admittance and thus prevent serious toxic effects by a sudden access of a large dose of poisonous material. Perhaps too the respiratory reflexes are of especial value in the case of the entrance of irritant particles, for the changes all result in decreasing the rate of admission of air and thus give greater time for the particles to fall on the mucous membrane, where they will be held.

SECTION 8. SOME EXPERIMENTS IN WHICH THE RATES OF DISTENSION AND COLLAPSE WERE INVESTIGATED.

In the experiments hitherto described we have been examining the total volume changes of a lobe of the lung which is inflated by the delivery of a large volume of air, the tracheal tube being provided with a big lateral outlet. The object aimed at by this method is to be sure that the maximum tracheal pressure at each inflation shall always reach the same height, so that no change in the result can be due to an alteration in the mechanical conditions of the distension. To attain this end the lateral exit from the trachea must possess very little resistance in comparison with that offered by the lungs. If in an experiment of this kind the record of the tracheal pressure changes be taken upon a rapidly moving surface (Fig. 29) it will be seen that the pressure rises fairly quickly and regularly, remains up for a very short time and then falls, the fall occupying only about one-half the time of the rise. Obviously, for many purposes this form of artificial respiration is not ideal, and this is especially the case if we wish to study the rate of distension and of collapse. For this purpose we should rather aim at inflating the lung with a constant pressure acting for a definite time. This can never be actually attained although we can approximate to it by conducting the artificial respiration differently. We have aimed at this in some experiments by rhythmically connecting the trachea, for a definite, short time, to a very large chamber containing air, constantly maintained at a fixed pressure. In expiration, the tracheal tube was freely opened to the external air, but, of course, the emptying was not effected in quite so satisfactory a manner, because the force producing it—the elasticity of the lung—is one which varies as the lobe empties.

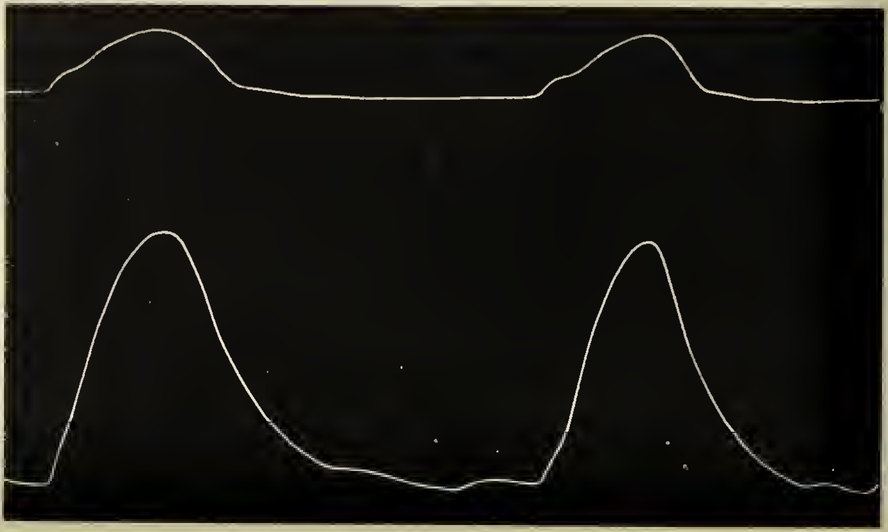

Fig. 29. Tracheal pressure (upper line) and lung volume tracings taken by the usual method but on a rapidly moving surface. (Surface moved by hand at about 48 mm. per second.)

Although the method is therefore only approximate, it yet offers certain advantages over the ordinary one. To apply it, it was necessary to employ a means of alternately connecting the trachea to the compressed air chamber and then to the external air, in regular rhythm. As in many instances it would further be of great advantage to be able to alter the duration of the filling and emptying, we devised the apparatus represented diagrammatically in Fig. 30. It consists of a short brass cylinder cut into three compartments by two perforated discs. Into each compartment a short wide tube opens, two, I and O, from one side of the cylinder, the other, *c*, from the opposite side. This latter tube is connected by a short piece of wide rubber tubing to a glass tube tied tightly into the trachea. The two ends of the cylinder are closed by two tambours, T_1 and T_2. When both tambours are undistended as in the figure, there is a free air passage from *c* through the two perforated discs to both I and O. If now the tambour T_1 be blown up, the rubber distends until it reaches the nearer disc, covers the holes in it, and thus closes the communication between I and *c* to all ordinary air pressures. Similarly the distension of the tambour T_2 closes the communication between O and *c*. The mode of working is as follows:—Starting with both tambours distended, the tube I is connected to a large reservoir containing air under pressure, the tube O being left open to the exterior. If now T_1 is suddenly deflated, air passes from I through *c* and distends the lungs until the pressure there is the same as in the main reservoir. T_1 is now again distended, thus closing the connection between the reservoir and the trachea. T_2 is next deflated and the lung at once empties itself through O. T_2 is then once more closed. The alternate distension and emptying of the tambours is effected rhythmically by a separate piece of apparatus which consists of two valves, one for each tambour. The valves are connected, respectively, to the two tubes *a* and *b* of the tambours and to a small reservoir of compressed air. When the valve is at rest the tambour is distended with air from the high-pressure reservoir. When the valve is depressed it first closes this connection, and then opens a passage to the exterior, and allows the tambour to empty. The rhythmic movement of the valve is effected by a sector fixed on a revolving axis driven at constant speed, so that by varying the length of the sector the time during which the tambour is empty is proportionately lengthened. In this way the time allowed for distension of the lobe and for its recoil can be conveniently and rapidly altered and the time intervals between the two can also be adjusted. The pressure in the high-pressure reservoir

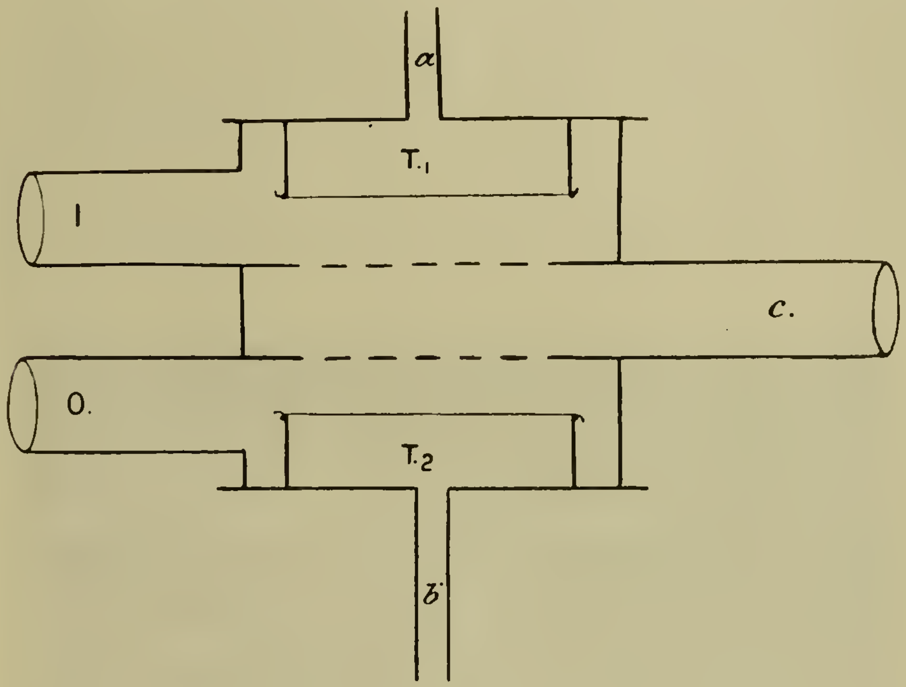

Fig. 30.

was kept up by the ordinary artificial respiration pump, and was maintained approximately constant by a mercury valve. The pressure used was about ten times that required for distension of the lungs. The pressure in the low-pressure reservoir was kept constant by hand, air being pumped into it by a pump whenever the pressure fell. This reservoir was of very large size so that the fall of pressure with each inflation was quite insignificant, and air was only pumped in during the intervals between two inspirations, *i.e.*, when the tambour T_1 was distended and blocked the connection to the lung. Further, the reservoir was provided with a water-manometer and water valve, and the tube connecting it to the tracheal cannula was of large diameter and as short as possible. A tracing, recorded upon a rapidly moving surface, in an experiment of this kind, is given in Fig. 31. In line A the air pressure used was 50 mm. of water. The increase in volume of

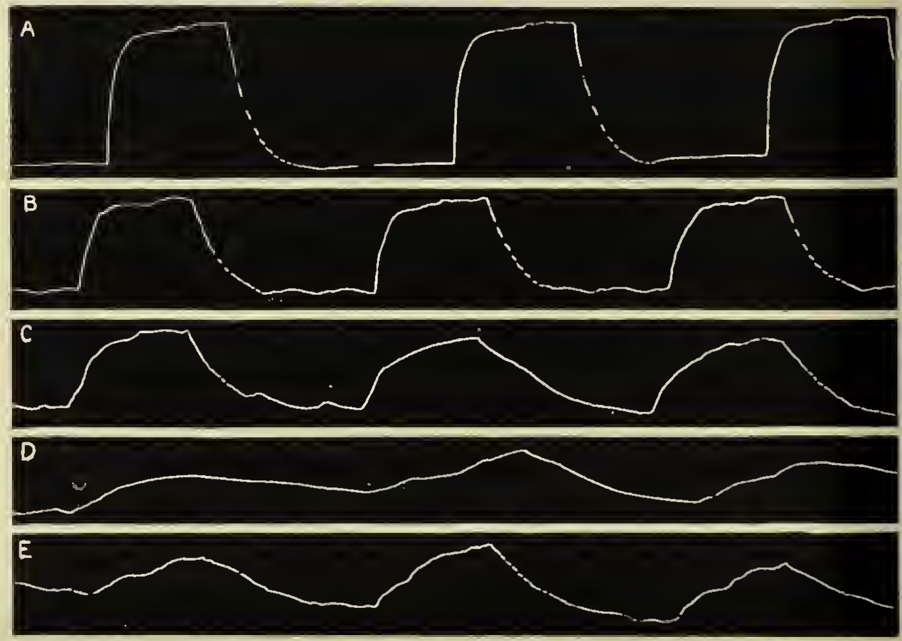

Fig. 31. Lung volume record only. In tracing A the pressure was 50 mm. of water and in the others 38 mm. In the four last the drum was moving at the rate of 15 mm. per sec.

the lobe is at first rapid but becomes very slow towards the end. The top of the curve is nearly flat, only showing a very slight tendency to rise. The fall in volume during expiration is much more gradual, especially in its later stages. The instant of opening of the valve, allowing the compressed air to enter the lung, is of course indicated by the sudden rise of the volume curve ; the instant of closure is marked by a slight rise in the curve seen on the summit. Similarly the end of the time during which the trachea has been open to the external air is marked by the slight notch on the lower horizontal line. In the second line, B, taken about 2 minutes later the distending pressure was reduced to 38 mm. of water, all the other conditions remaining unchanged. The general features of the record are the same as before, only differing in that distension and contraction are a little less rapid and that the successive volume changes are not quite so uniform. The latter difference is due to the low pressure employed, which, in a collapsed lobe, is only sufficient to produce slight distension, and it is easy to see, therefore, that if several of the alveoli are collapsed they will not be opened so promptly as by the higher pressure. The lung therefore will not behave exactly as a distensible elastic sac, some of the distending force being used up at the start to separate the alveolar walls. This adhesion retards free inflation more easily if the force employed is only a little above that required to start the distension.

In this condition the lobe is however in the best and most sensitive state to show any further impediment to the entrance of air such as an increase in resistance in the bronchioles.

The result of excitation of the vagus, upon a lobe under these conditions, is given in the next three records of this figure. Line C followed immediately after line B, and gives the first three inflations after the commencement of the excitation. The effect is most marked, and shows itself both in the rate of distension and contraction, but especially in the latter. The second distension after the commencement of the stimulus, shows the change most markedly, but the third line D shows a tendency to recovery, a condition frequently observed when experimenting upon cats. The fourth and fifth waves are very small, and are affected in rate of both rise and fall. Line E gives the three waves following the cessation of stimulation, and shows the recovery in a very marked way. In several of these waves we see that the curve continues to rise although the tambour has closed the connexion between the distending pressure and the trachea. This rise is unquestionably due to the fact that the distending pressure had not acted long enough to completely distend the alveoli to that pressure, and thus when the tracheal orifice was closed the pressure in the trachea was still higher than that in the alveoli, and the latter were therefore still further distended. The effect of vagal excitation as studied in this method very clearly brings out the marked effect upon emptying. In other words, the act of filling even when this low pressure is employed is effected much more efficiently than the act of emptying.

It is obvious that this method of experimenting can be of great service when studying the distensibility of the alveoli under different conditions. The method has also proved of considerable service, in that by it we could conveniently watch the behaviour of the lung when varying the times allowed for distension or for collapse. It has proved especially useful in studying the production of over-distension, and was for instance the method employed when taking Figs. 16 and 17, p. 128.

SECTION 9. EFFECTS OBTAINED AFTER THE DEATH OF THE ANIMAL.

Many of the earlier workers upon the bronchial muscles obtained indications of bronchial constriction in animals which had been recently killed. We have therefore made a few observations of this nature, usually at the end of an ordinary experiment. These were mainly of

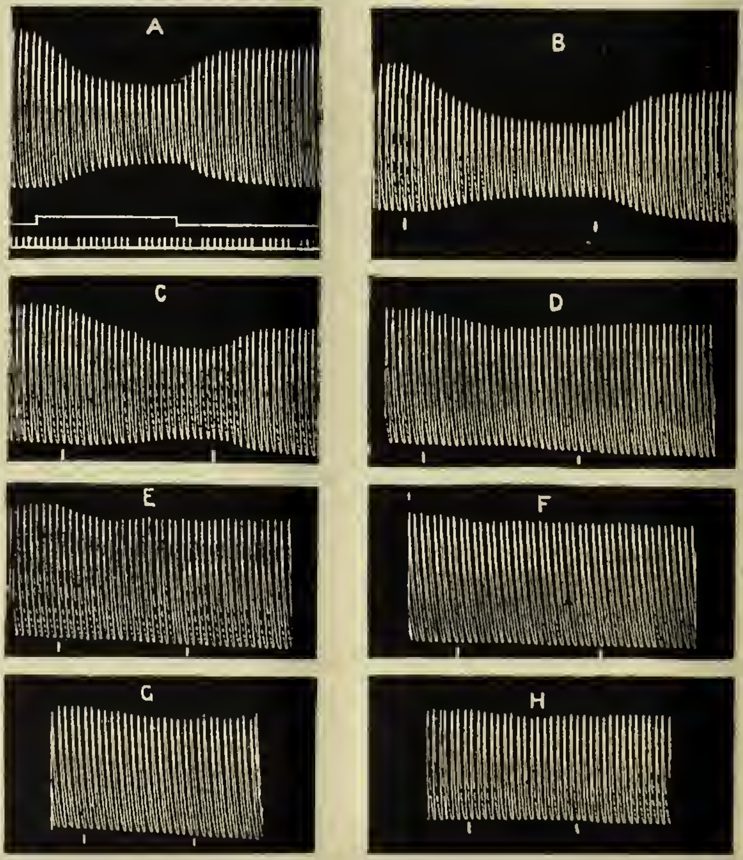

Fig. 32. Rabbit. A series of excitations of the vagus taken after the animal had been bled and the heart excised. A immediately after, B 5 mins., C 10 mins., D 14 mins., E 16 mins., F 20 mins., G 24 mins. and H 28 mins. after.

two kinds. In the first we studied the action of the vagus post-mortem, and in the second, recorded changes which gradually developed spontaneously after death. In Fig. 32 we reproduce the results of a series of excitations of the vagus. In this experiment a rabbit was pithed, and a series of observations, some of which have been given in Figs. 11 and 22, recorded. The animal was then bled from the carotid artery, and the series of records given in Fig. 32 obtained. Tracing A was taken immediately after death, and should be compared with that of Fig. 11, which had been obtained some time previously from the same animal. The stimulus was a weak one, but a perfectly typical bronchial constriction was obtained. Tracing B was obtained 5 minutes later, and only differs from the first in that the onset of the constriction was somewhat slower. C was taken 10 minutes after death, and already the effect is very much weakened, the greatest difference being a still slower development of the constriction than in B. Records were then taken every 2 minutes, and some of these are given. Thus D was taken 14 minutes, E 16 minutes, F 20, G 24, and H 28 minutes after. They show a gradual diminution of effect, but there is still a slight contraction even in the last. The vagus therefore can cause some contraction of the bronchioles half-an-hour after the death of the animal. The nerve terminals are still excitable for a longer period, for pilocarpine caused a very pronounced contraction a few minutes after electrical excitation of the vagus had ceased to produce an effect. It is noteworthy that in this animal very little post-mortem contraction had occurred 30 minutes after death, though as a rule this is very pronounced at a much earlier time.

In a further series of observations the animal was killed by bleeding, and the volume record continued for some time after death. In all instances a slow and prolonged constriction of the bronchioles was recorded. This usually commenced within a minute after the death of the animal, and became so marked as to nearly occlude the bronchioles completely. In a few instances this was followed by relaxation, but in most cases the constriction persisted during the whole time of observation, which in some instances amounted to more than an hour. In Fig. 33 are reproduced short lengths of the tracing taken in one of these experiments. The maximum constriction was recorded 7 minutes after death, and then relaxation commenced and was complete 41 minutes after death. No further contraction was recorded. Fig. 34 shows another instance which is of interest, because the course of events differs from any of the others we have observed, in that there is a preliminary constriction followed by relaxation and a subsequent second constriction. Maximum constriction was attained in this instance in

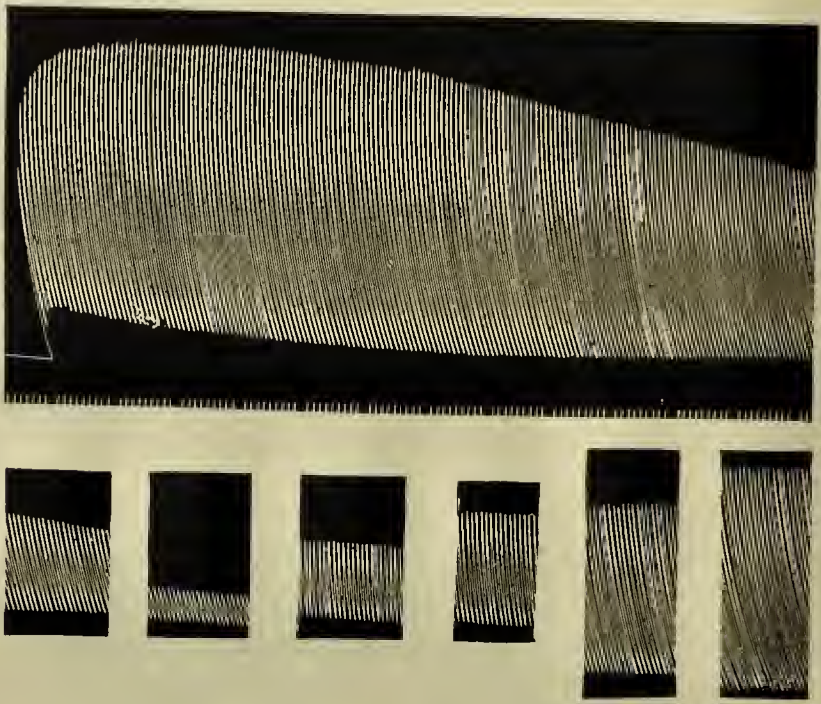

Fig. 33. Cat. Record of air movements after death by bleeding. The later short pieces of tracing are at intervals of 2 mins., with the exception of the last, which followed an interval of an hour.

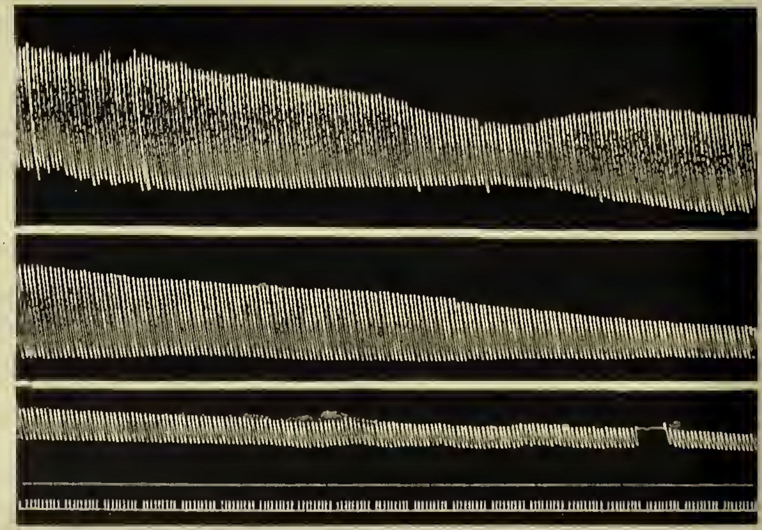

Fig. 34. Cat. A.C.E. Post-mortem contraction of the bronchioles: the three lines follow one another in succession.

10 minutes, and no relaxation occurred. In other cases the course of the contraction was much slower, only reaching its maximum in from 20 to 40 minutes. We think the cause of this contraction is asphyxial.

In a few experiments we volumetrically recorded the air movements of a lobe of an excised lung artificially perfused with blood at constant pressure. Such lungs did not show the contraction just described. These experiments are again referred to in the section dealing with the action of drugs.

SECTION 10. ACTION OF DRUGS UPON THE BRONCHIAL MUSCLES.

I. *Muscarine and Pilocarpine.* The action of these two alkaloids upon the bronchial muscles is so similar that it will be convenient to consider them together. They may be used as types of that class which produces bronchial constriction. The discoverers of muscarine, Schmiedeberg and Koppe[1], observed the marked dyspnœa which followed its injection, but ascribed it to an action upon the respiratory centre. Brunton[2] relying upon observation of the exposed lungs and heart, considered that the dyspnœa was due to constriction of the pulmonary arteries, for he observed that the lung became paler in colour, the right side of the heart engorged, and the left empty. This cannot however be the true explanation, for it has been shown that there is no rise of the pulmonary blood-pressure, whereas there is one of the pulmonary venous pressure. (Grossmann, confirmed by ourselves.) We consider the true explanation of the colour change to be partly that the amount of blood in the lung is decreased, just as we have seen is the case in cardiac inhibition produced by vagal excitation, and partly that the contained blood becomes venous, and thus when seen in thin layers the depth of colour is less pronounced than if it were arterial.

Grossmann[3] studied the action of muscarine upon the lungs more particularly with respect to the mode of production of the œdema which he describes as following the injection. He concluded that the whole of the effects were of cardiac origin. He found that the pulmonary venous pressure rose considerably and that the pulmonary arterial pressure at first fell and later rose. From these results he argued that a considerable congestion of the lung capillaries is produced, which leads to "Lungenstarrheit," and thus to a great diminution of distensibility with its consequent dyspnœa. Against this we must argue that in our hands muscarine has usually caused a very marked fall in the pulmonary pressure, and that our direct volumetric examination of the blood-volume of the lung has shown that the amount of blood does not as a rule increase but remains practically constant, and if a change is produced it is usually a diminution. Again, "Lungenstarrheit" cannot possibly explain the great hindrance to the entrance of air into the lungs, which is always observed. Even by producing a most marked congestion, as by temporarily clamping the pulmonary vein issuing from a lobe, the restriction in the air movements of that lobe is inconsiderable, and altogether out of proportion to the marked effect on the air movements produced by a small dose of muscarine. We may further point out that in the curves Grossmann published, the development of the dyspnœa takes place during the early marked cardiac inhibition caused by the drug, *i.e.* at a time when the pulmonary artery pressure is extremely low.

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[1] Schmiedeberg and Koppe. *Das Muscarin.* Leipzig, 1869.

[2] Brunton. *Brit. Med. Journ.* Nov. 14, 1874.

[3] Grossmann. "Das Muscarin-lungenöden." *Ztschr. f. klin. Med.* XII. p. 550. 1887.

In a later paper Grossmann[1] takes into account a possible effect upon the bronchial muscles, but still considers that the main cause of the dyspnœa is due to the action of the drug upon the heart and that the bronchial constriction only plays a subordinate part.

It is well known that muscarine and pilocarpine produce tonic contraction of involuntary muscle (*e.g.* stomach, intestines, bladder, etc.) throughout the body, and our experiments show that the bronchial muscles are no exception to this rule. A small intravenous injection of muscarine produces after a very short latent period a rapid diminution in the quantity of air passing in and out of the lung, and if the force of inflation is not too great and the amount injected sufficient, a complete block is set up so that no air can either enter or leave. This effect is perfectly shown in Fig. 35, where 0·001 grm. muscarine was injected at the indicated mark. The movements of the recording lever after the muscarine has produced its effect are purely vascular.

During this action the animal tends to become asphyxiated, and to prevent death it is necessary to increase the force of inflation. The effect of this is seen in the same figure. When the force is sufficiently great the block is overcome and more air passes into the lung, but the elastic recoil of the lung is unable at first to expel this, and the lobe becomes rapidly over-distended.

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[1] Grossmann. *Ztschr. f. klin. Med.* XVI. pp. 161 and 270. 1889.

In many cases the bronchial constriction produced by muscarine or pilocarpine is attained much more gradually, and the latent period up to the time of complete obliteration of the air movements may extend to even two or three minutes, although such a very prolonged period is exceptional. In these cases the constriction often occurs as a series of waves of alternating contraction and dilatation, a condition shown in Fig. 36, where, although some contraction occurs soon after injection, complete obliteration is not attained until after a latent period of a minute and a half from the time of administration.

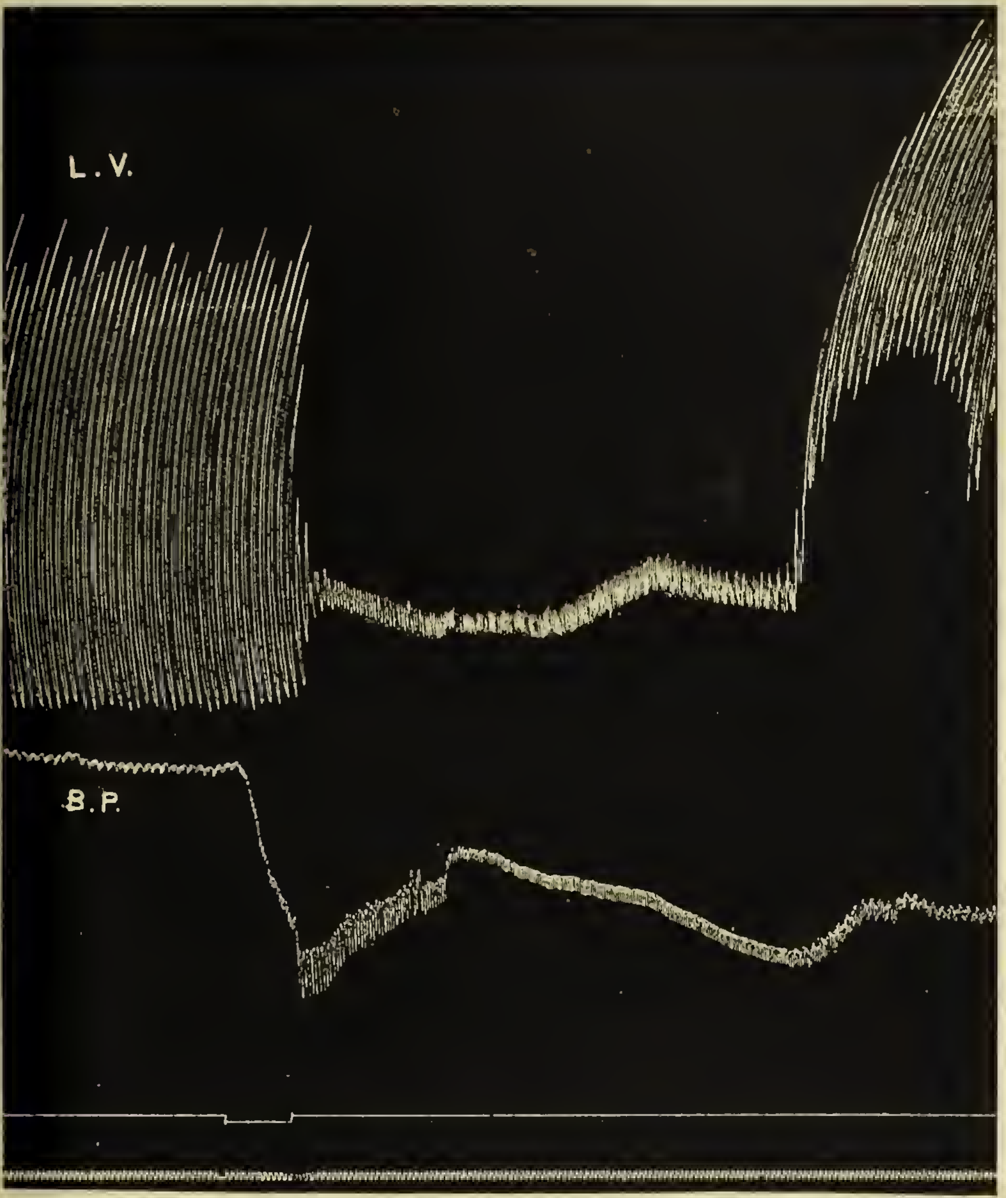

Fig. 35. Cat. A.C.E. Injection of 0·001 grm. muscarine. Reduced $\frac{1}{3}$.

Quite commonly, instead of producing immediate constriction, muscarine gives rise to an initial and well-defined dilatation, which is rapidly followed by the more important constriction : this is clearly shown in Fig. 37, in which the over-distension of the lung which obtains with the gradually increasing bronchial constriction is also well illustrated. This initial relaxation is not peculiar to bronchial muscle but can also be frequently observed in the muscle of the intestines and bladder after injections of pilocarpine.

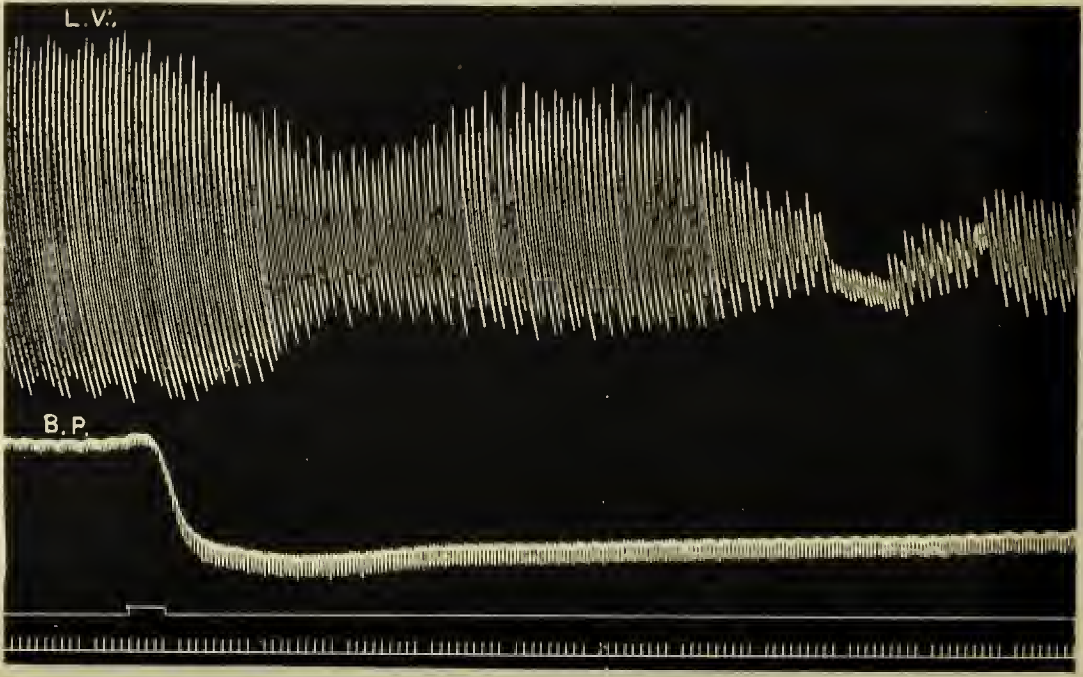

Fig. 36. × $\frac{1}{3}$. Cat. A.C.E. Right vagus cut. Shows the effect of injecting 0·0075 grm. pilocarpine nitrate.

The cause of this preliminary dilatation may be, either (i) diminution in the amount of blood in the pulmonary capillaries, thus causing greater distensibility, or (ii) relaxation of the bronchial muscles. We do not think the first can completely account for the effect, for it is sometimes very marked, and at other times it is absent although the vascular changes are produced as usual. The true explanation probably is, that the drug excites the broncho-dilator fibres as well as the broncho-constrictor, and that the former react more quickly or more powerfully at first, and the reason the effect is not always present is that tonic constriction of the bronchioles is as a rule absent.

In all these experiments A.C.E. or ether were the anæsthetics usually employed. In a few cases the experiments were performed on pithed animals when identical results were obtained.

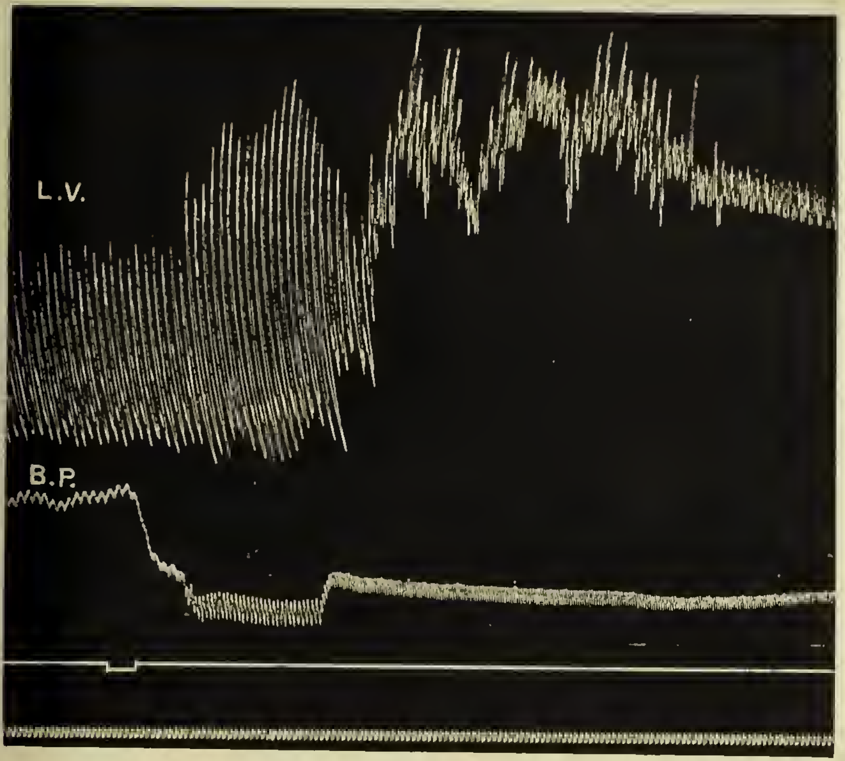

Fig. 37. × ⅓. Cat. Urethane. Shows effect of 0·001 grm. muscarine.

That this broncho-motor effect is peripheral was conclusively shown by perfusion experiments. The heart and lungs were taken from a cat and defibrinated blood at the body temperature was then perfused through the pulmonary artery, warm air being simultaneously and regularly blown into the lungs by the respiration pump: a lobe of one lung was made to record its volume in the usual manner. The addition of a small quantity of muscarine to the perfusing fluid produced an immediate constriction of the bronchioles. The results thus obtained clearly show that "Lungenstarrheit" can have little or nothing to do with the production of the effect.

In most experiments, the animal experimented upon was the cat, but in many the dog and rabbit have been used, and the results in all respects agreed with those above described.

II. *On certain conditions which modify the action of the drugs which produce broncho-constriction.* After section of both vagi muscarine still produces its normal effect. The vexed question as to whether muscarine and pilocarpine can produce any effect on involuntary muscle after the administration of atropine, seems to depend, at least as far as bronchial muscle is concerned, on the amount of atropine employed. After injections which are sufficient to paralyse the vagi, large injections of muscarine are still able to produce a very slight constriction, but after large atropine injections (0·08 grm. in the case of a medium-sized cat) no amount of muscarine has any effect. Fig. 38 shows the result of the administration of 0·004 grm. of muscarine to a cat which had previously received an injection of 0·0055 grm. atropine; some bronchial constriction is apparent.

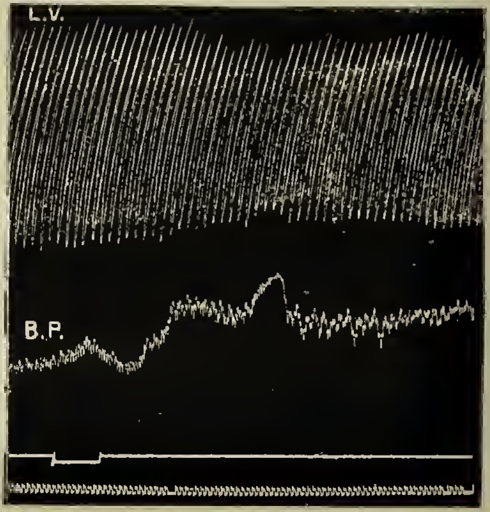

Fig. 38. × $\frac{1}{3}$. Cat. Urethane. Shows effect of injecting 0·004 grm. muscarine after atropine.

In some of our experiments urethane was employed as the anæsthetic; in these a distinct difference in the response of the bronchial muscles to muscarine could be determined in most cases. Not uncommonly, after complete anæsthesia with urethane, moderate injections of muscarine failed to produce any constriction, and larger injections only did so after a much prolonged latent period. Fig. 39 shows the effect of muscarine on an animal anæsthetised with urethane, where it will be observed that it is not till after a two minutes interval that any very marked constriction is induced, and this is no exceptional case, indeed sometimes a latent period of five minutes has been noted. Urethane also behaves as a complete antidote to muscarine as regards the involuntary muscle of the alimentary canal. The antagonism of urethane to muscarine is nothing like so complete with bronchial muscle as with intestinal, and its action in small amounts is rather to delay the muscarine effect than to eliminate it.

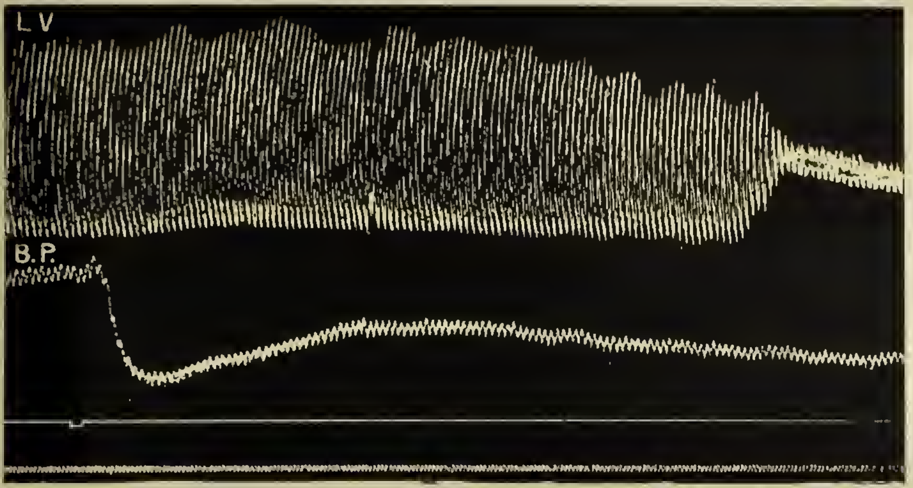

Fig. 39. $\times \frac{1}{2}$. Cat. Urethane. Injection of 0·001 grm. muscarine.

Curarised animals behave very similarly to non-curarised as regards their response to muscarine, in some cases, however, the latent period is slightly prolonged. Fig. 40 shows this condition.

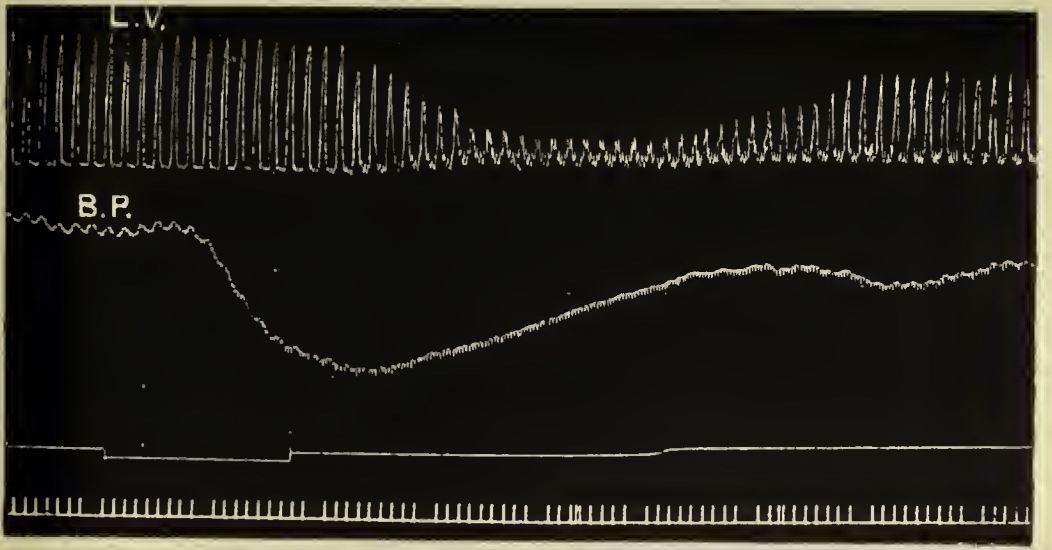

Fig. 40. $\times \frac{1}{3}$. Cat. A.C.E. Curare. Injection of 0·001 grm. muscarine.

III. *The effect of atropine after muscarine.* If atropine is injected into an animal in which moderate broncho-constriction has been induced as the result of a previous injection of muscarine or pilocarpine, an immediate dilatation usually results; this is well shown in Fig. 41. If however the bronchi are so constricted as to produce a complete block to the air of inflation, the effect of atropine is only seen after a more or less considerable latent period: the explanation of this being that when a complete state of collapse is produced by bronchial constriction, the lobe in the oncometer does not expand directly the constriction passes off but requires a rather higher pressure to produce the initial expansion, a condition we have already discussed (p. 130). Fig. 42 shows the effect of atropine in such a condition. Before the injection there is a markedly slow heart and no air enters or leaves the experimental lobe—the movements recorded being purely vascular. For about 50 seconds after the injection no effect is produced except the one on the heart, but after this interval air suddenly enters the lung, and as this is repeated three or four times while no air escapes the lungs become gradually over-distended. In about 80 seconds from the time of injection a considerable amount of air has been driven into the chest and the elastic recoil of the distended lungs suddenly over-

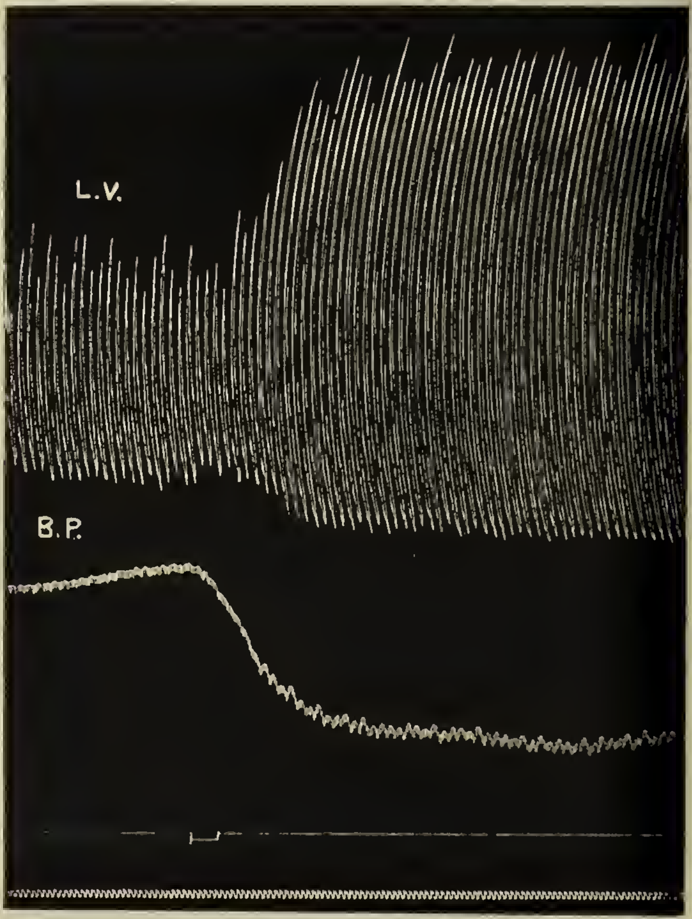

Fig. 41. × $\frac{1}{3}$. Cat. A.C.E. Injection of 0·01 grm. atropine sulphate after muscarine.

comes the resistance due to the bronchial constriction; an expiration results, which is the prelude to a very rapid recovery. In many cases the latent period between the administration of atropine and recovery is greater than that shown in the Figure and may reach three minutes or even longer.

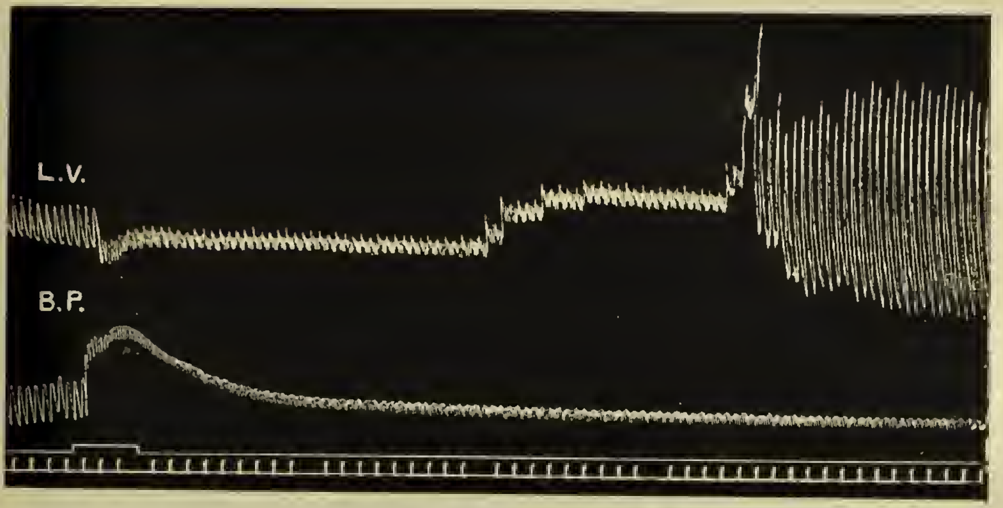

Fig. 42. × $\frac{1}{3}$. Cat. Urethane. Injection of 0·01 grm. atropine sulphate after muscarine.

In only one case in our experiments did atropine produce any initial constriction of the bronchi; this was in a cat which had been anæsthetised with urethane.

IV. *The action of some other drugs on the bronchioles.* These will be briefly considered, and for the convenience of description it will be advantageous to divide them into groups.

Broncho-constrictors.

A, acting on nerve-endings.	Muscarine, pilocarpine.
	Digitalin.
	Neurine.
	Aspidiospermine.
B, acting on muscle.	Barium chloride.
	Veratrine.
	Salts of many heavy metals.
	Bromine.
C, central and peripheral.	CO_2.

Broncho-dilators.	
	Atropine, hyoscyamine, hyoscine.
	Chloroform and ether.
	Urethane.
	Hydrocyanic acid.
Dilatation generally preceded by slight constriction.	
	Lobelia, nicotine.
	Curare.
	* * *
	Morphine.
Relatively inactive.	Suprarenal extract.
	Ergot and its constituents.

Physostigmine is one of the most active of the broncho-constrictors; in very small amounts it is able to produce big effects, for example 0·0005 grm. injected into the jugular vein of a cat produced well-marked constriction, almost leading to complete cessation of air movements. The latent period after injection is usually a little longer than that of muscarine and the constriction frequently begins as a series of waves, which, becoming more and more pronounced, culminate in tonic contraction and obliteration of all air movements. The effect is still obtained after section of the vagi and after moderate injections of atropine. Physostigmine can still produce a contraction of the bronchi in animals which have received so much atropine that muscarine has no effect. After very large doses of atropine, however, physostigmine produces no broncho-constriction although drugs which act on muscle directly such as barium still give rise to constriction. We therefore believe that physostigmine acts on the peripheral vagal endings, but that its antagonism to atropine is much more complete than is the case with muscarine. This effect harmonises with the action of physostigmine on the iris.

Digitalin (Merck) produces a very rapid broncho-constrictor effect and complete occlusion may occur. The reaction is not produced in the atropinised animal (*i.e.* one in which the vagi are paralysed to electrical excitation) to the same extent, although some slight constriction still commonly occurs. Digitalis is not a suitable drug with which to demonstrate bronchial constriction on account of its powerful effect on the heart, for a dose sufficient to produce well-marked bronchial constriction is frequently sufficient to kill the animal.

Neurine gives rise to an immediate and decisive constriction of the bronchial muscles although by no means of so powerful a type as that observed after muscarine. We consider it possible that the exacerbation of the respiration noted by Halliburton and Mott, and others, when small amounts of neurine are injected into the circulation, may be explained in this manner. The constriction is very persistent but is not produced in the atropinised animal.

Barium chloride is taken as a typical example of a drug which produces its effect by acting directly on plain muscle. Small injections lead to a slow increase in blood-pressure, partly cardiac, but mainly vasomotor, and associated with this is a gradual constriction of the bronchial muscles, ultimately attaining to the well-defined asthmatic condition. This effect can be obtained equally well on the atropinised animal. Fig. 43 is an experiment on a cat with vagi cut. The lungs are being inflated from a large reservoir at a constant pressure. (*a*) Shows the normal condition of affairs. (*b*) The condition one minute after the intravenous injection of 3 c.c. of 1 °/₀ $BaCl_2$: the blood-pressure is now much higher, the residual air in the enclosed lobe is increased, and less air enters and leaves during each respiration. This condition is developed slower than with drugs acting on nerve, but is much more persistent.

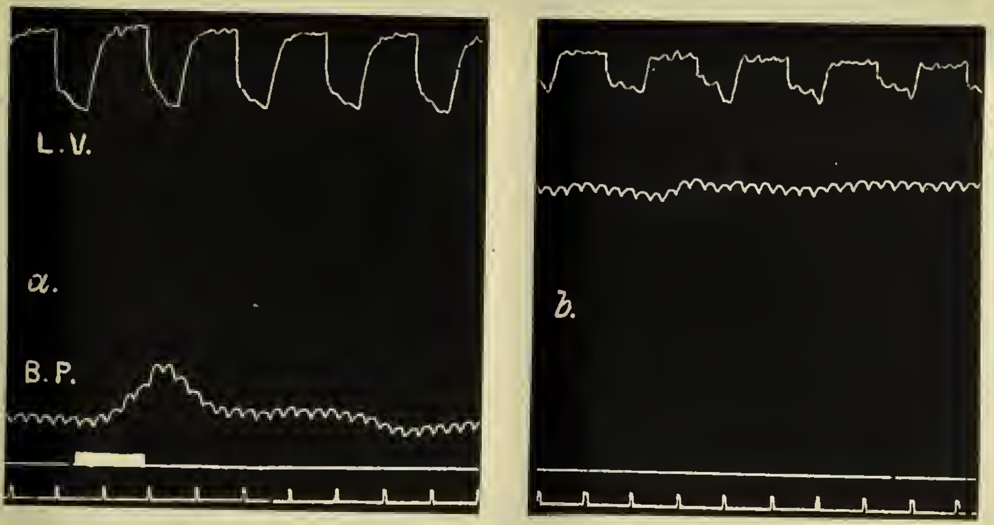

Fig. 43. × ⅓. Cat. A.C.E. Vagi cut. Tracing by method described on p. 148. Zero B.P. 35 mm. below abscissa line. Injection of 0·03 grm. $BaCl_2$.

Veratrine has an effect similar to barium; 1 c.c. of a 0·1 °/₀ solution will give rise to typical constriction.

Gold chloride. Most of the heavy metals when injected directly into the circulation give rise to increased movements of plain muscle throughout the body. These effects are not however usually obtained when the metallic ion is administered by the mouth because, with the exception perhaps of arsenic, mercury and lead, so very little is absorbed. Gold chloride is taken as an example of this class. When it is injected into a vein a slow but clearly defined constriction of the bronchioles developes. It rarely obliterates the air movements and is never of so intense a character as that seen after barium. The effect is probably mainly on plain muscle and not on the nervous mechanism.

Bromine. This drug also produces contraction of the bronchioles whether administered by inhalation of the vapour or by injection of a weak saline solution. According to the dose given the constriction may be slight and transitory (Fig. 44), or may lead to complete occlusion from which recovery is extremely slow. It acts peripherally and upon the muscle fibres, for it is produced equally as well if both vagi be divided, or after a large dose of atropine. Bromine we know powerfully excites nerve endings and then kills them, but whether in these cases of bronchial constriction the drug also excites the nerve endings we are not able to say.

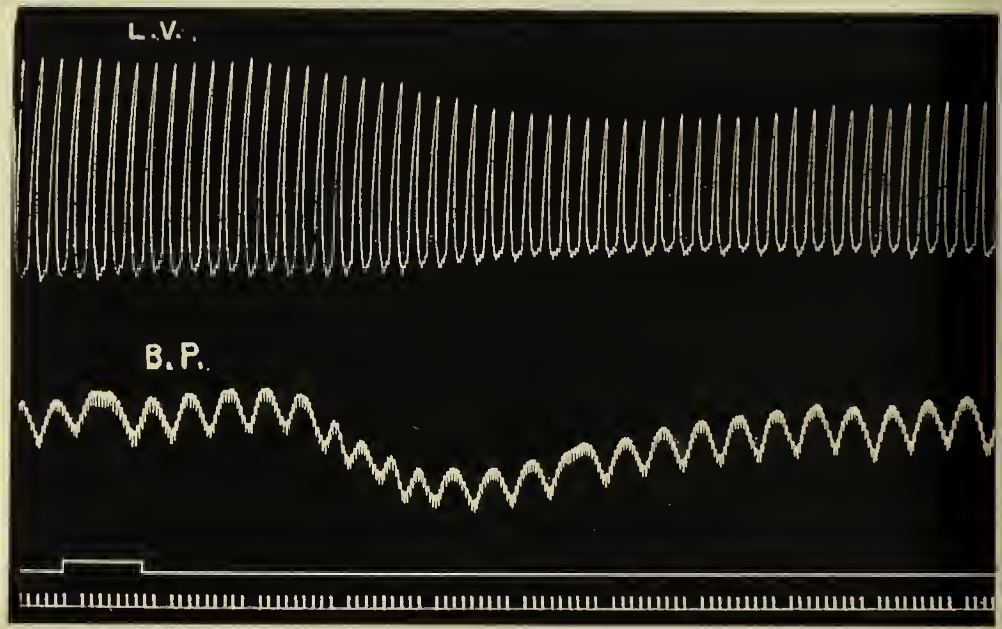

Fig. 44. Cat. A.C.E. Injection of 3 c.c. of a dilute solution of bromine in saline solution. Zero B.P. 49 mm. below abscissa line. Reduced $\frac{1}{3}$.

Carbonic acid. The inhalation of carbonic acid gas has already been shown by Einthoven[1] to lead to a tonic constriction of the bronchi. He showed that in his experiments this was brought about through the medullary centre, for the increased tonus disappeared on division of the vagi in the neck. Einthoven therefore concluded that the gas acted directly upon the centre in the medulla, but did not consider the possibility that the result might have been reflexly produced through excitation of the nerve terminals of some afferent fibres of the vagus (*e.g.* the pulmonary). Einthoven found that no contraction was produced if both vagi had been divided, even if pure carbonic acid were used. We can, on the whole, confirm Einthoven's results, but in one experiment, in which fairly concentrated carbonic acid was used, we observed marked constriction which was not abolished by section of the vagus upon that side (Fig. 45). In connection with this figure we must point out that we have frequently proved that vascular changes such as are indicated in that figure are not sufficient of themselves to account for the marked air changes produced. We conclude therefore that carbonic acid as a rule acts by excitation of the centre in the medulla but whether directly or reflexly we are not yet in a position to definitely decide. In some cases the gas can act peripherally, but whether upon nerve or muscle we have no evidence.

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[1] Einthoven. *loc. cit.* p. 423.

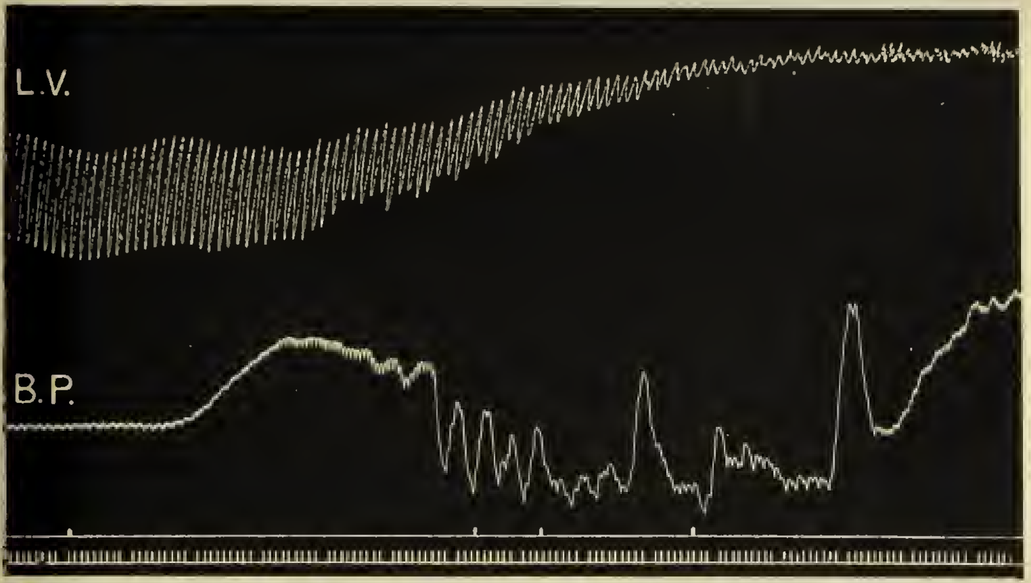

Fig. 45. Cat. Ether. At the first signal a strong dose of CO_2 was added to the respired air, at the second the right vagus was tied, at the third cut, and at the fourth the administration of CO_2 was stopped. Zero of B.P. 27 mm. below abscissa line. Reduced nearly ½.

Atropine, hyoscyamine, and hyoscine all act in the same way. The action of atropine has already been discussed. It only remains to add that in the cat atropine gives rise to no dilatation unless some initial constriction has been induced, there being apparently little or no normal tonus of the bronchioles in this animal. Dreser first conclusively showed that stimulation of the pneumogastrics did not cause contraction of bronchial muscle after atropine.

It is not our intention here to deal with the treatment of spasmodic asthma, the radical cure of which consists in removing the source of peripheral irritation; but we desire to point out the impossibility of an attack whilst the patient is under the influence of atropine. During an attack an injection of atropine will quickly abort it. This is of course well recognized by clinicians and the inhalation of strammonium fumes forms the routine method of treatment.

Chloroform and ether produce some dilatation of bronchioles; the effect is never of a very marked character, but can usually be seen in the lightly anæsthetised animal by suddenly increasing the supply of anæsthetic. To get a good result it is necessary to first produce some slight artificial constriction. This we believe is probably an action on the vagal nerve-endings since after a large dose of anæsthetic excitation of the vagus gives either an imperfect or no constriction. The effect of anæsthetics has received further discussion on p. 133.

Urethane. In a number of our experiments we used urethane as an anæsthetic, and it was only after many trials that we discovered that this substance, though not affecting the vagal endings in the heart nor the cardiac muscle, had a pronounced effect on ordinary plain muscle and acted as an antidote to muscarine. This drug produces some dilatation of the bronchioles, if administered to animals showing increased bronchial tonus as, for example, after a dose of muscarine.

Hydrocyanic acid. This substance was employed on account of its marked stimulant effect on the medulla, and it was therefore considered possible that it might produce some constriction by direct stimulation of the vagal centre. Such however has not been found to be the case nor have we succeeded in finding any substance which will produce this result with the exception of CO_2, although the effect may be brought about readily enough reflexly by such substances as veratrine acting on the peripheral ends of the fifth nerve.

Small inhalations or injections of HCN produce a gradual relaxation of the involuntary muscle which is peripheral and probably due to a weakening of both nerves and muscles.

Nicotine has an excito-motor effect on bronchial muscle resulting in constriction, which however soon gives place to dilatation. Initial dilatation with small doses of nicotine is not usual, but when large quantities are injected dilatation may occur immediately, and this is especially the case after the previous injection of small quantities of some broncho-constrictor body. Fig. 46 shows a typical effect where the upper tracing represents the intestinal volume, the second the air volume of the lobe of the lung, and the lowest the blood-pressure. The lung undergoes a considerable increase in blood-volume as shown by the rise in the lower limiting line. Distinct constriction of bronchi can be seen which is beginning to pass off on the right of the tracing.

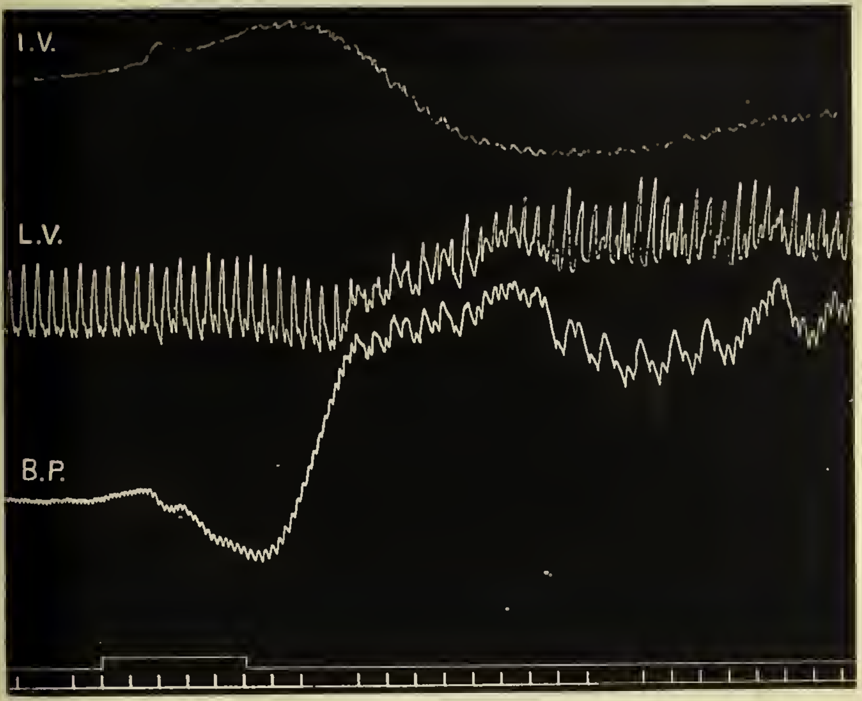

Fig. 46. Cat. Urethane. Injection of 0·003 grms. nicotine. Zero of B.P. is 20 mm. below the abscissa line. Reduced $\frac{1}{3}$.

Lobelia was tried in a few cases on account of its reputation as a specific in spasmodic asthma. Dreser[1] found that after lobelia, stimulation of the vagi no longer narrowed the calibre of the air passages. We employed two preparations, (1) lobeline, which we found inactive, (2) the ethereal tincture (B.P.). This latter was mixed with half its volume of normal saline, the ether evaporated off, and the solution then filtered. This solution produces a marked rise in blood-pressure, and some slight constriction of bronchi. But if the bronchial tubes are at all constricted, lobelia produces a quick dilatation which rapidly passes off, and the original constriction returns and may be dispelled by another injection of lobelia. Fig. 47 shows such a condition where the bronchi were first constricted by means of pilocarpine, and the lobelia is here seen to induce almost instantaneous dilatation, which however soon dies away. A second injection is shown on the same tracing. The effect may be repeated many times, and recalls the dilatation following vagal

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[1] Dreser. *Arch. f. exp. Path. u. Pharm.* xxvi. p. 237. 1890.

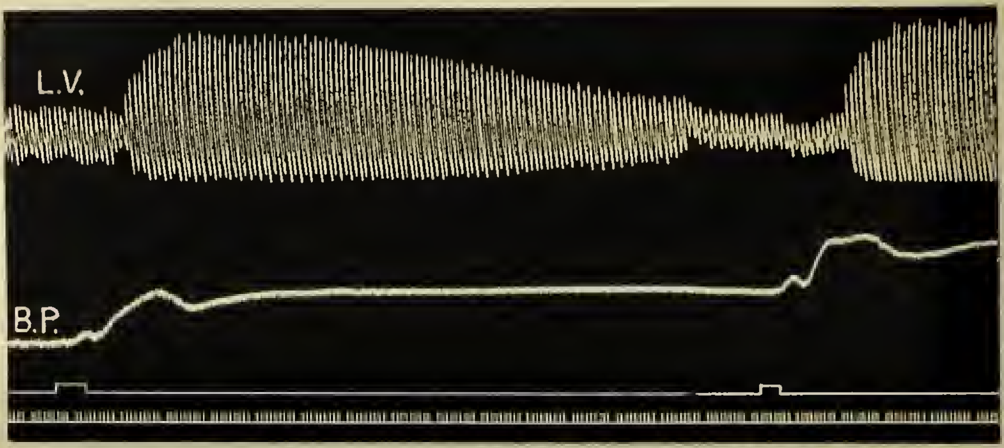

Fig. 47. Cat. A.C.E. Injection of two successive doses of lobelia prepared as described in the text. Zero of B.P. is 25 mm. below the abscissa line. Reduced ½.

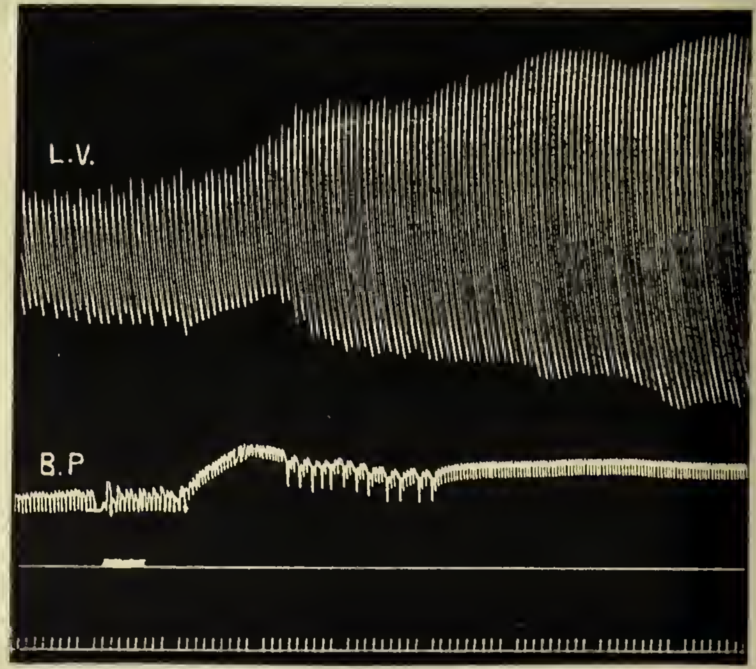

Fig. 48. Cat. A.C.E. Injection of 1 c.c. of a 1 % solution of curare. Pilocarpine has been previously administered. The zero of B.P. is 20 mm. below the abscissa line. Reduced ½.

excitation after pilocarpine. This effect is in marked contrast to atropine, where the dilatation is absolutely permanent.

Curari has an action on the bronchi agreeing in the main with those of nicotine and lobelia. If it be injected into the vein of a normal animal there is slight and gradual constriction. This effect is probably analogous to the increased splenic movements noted by Schäfer and Moore and the stimulation of the intestinal movements described by Nasse. When administered to an animal in which the bronchi are already in a condition of some tonus, well-defined dilatation results (Fig. 48). This effect is not permanent, and the bronchi assume their previous condition in 2 or 3 minutes. Curari in no way prevents an injection of muscarine producing its full effects. Its action is produced equally well after the section of the vagi.

Morphine. Large injections in the case of the normal cat lead to very considerable constriction of the bronchial muscle after an initial transitory small dilatation, and this after the vagi are cut. Small doses, on the other hand, are followed by a slight dilatation only, which is the better marked if the bronchi are in a condition of increased tonus. Fig. 49 shows the effect of injecting into the jugular vein of a cat 4 c.c. of a 2 % solution of morphine. The injection caused a great fall of blood-pressure.

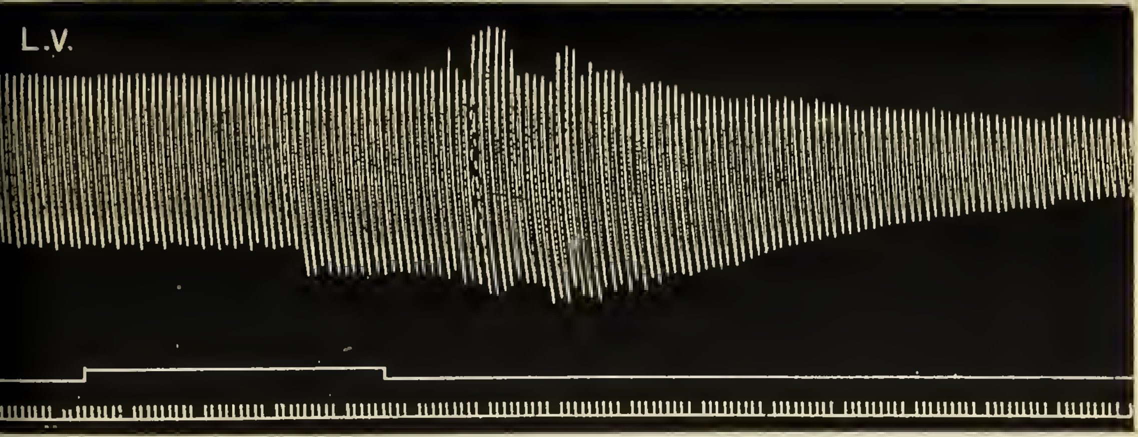

Fig. 49. Cat. A.C.E. Injection of 0·080 grms. morphine acetate.

Suprarenal extract produces very little effect on the bronchi, small doses generally giving rise to neither constriction nor dilatation. With large injections, however, constriction of a temporary character has in a small percentage of cases been observed. It is possible that in this case the free movements of the air are interfered with by the great vascular engorgement which is present.

Ergot. We have employed two preparations of this substance, (1) commercial ergotin, which probably contains all the active ingredients present in the fungus, and (2) sphacelotoxin of Jacobj—the gangrene-producing body. In no case was any immediate effect observed on the bronchi. The ergotin produces an immediate fall of blood-pressure associated with splanchnic dilatation. The calibre of the bronchioles remains apparently unaltered, but increase in the blood-content of the lung is evidenced by a rise in the lower limiting line.

CONCLUSIONS.

1. The degree of contraction of the bronchioles can only be satisfactorily demonstrated by some method which directly or indirectly estimates the volume of air passing through them. The plethysmographic method fulfils this condition.
2. The broncho-constrictor fibres run in the vagus; none are found in the sympathetic: there is no central tonic effect.
3. Contraction of the bronchioles may lead to collapse or overdistension of the lung, depending upon the force of the inflation on the one hand, and the time allowed for deflation on the other.
4. In the dog and rabbit, typical bronchiolar constriction immediately follows vagal stimulation. In the cat, the common response is a preliminary slight constriction followed by recovery, and the typical constriction only comes on some seconds after the stimulus has ceased.
5. The vagus contains broncho-dilator fibres, as well as broncho-constrictor, and the former are particularly well developed in the cat, thus yielding an explanation of the anomalous results seen in this animal.
6. The sympathetic contains no broncho-dilator fibres.
7. The presence of broncho-dilator fibres is most readily demonstrated by excitation of the vagus, after an artificial tonus has been set up by means of muscarine, pilocarpine, etc. The broncho-dilatation dies away soon after the stimulus ceases.
8. Both the constrictor and dilator fibres in the vagus supply the lung on the same side only.
9. The inhalation of ether or chloroform for anæsthetic purposes abolishes the effect of the vagus on the bronchioles. This is due to the paralysis of the nerve-endings by direct absorption through the mucous membrane.

10. Excitation of the vagus leads to a diminution in amount of the blood in the lungs. This produces increased distensibility—diminished "Lungenstarrheit" in v. Basch's sense.

11. Reflex bronchiolar constriction is best obtained by exciting the nasal mucous membrane. Little or no result has been obtained by stimulating the sciatic, central vagus, superior laryngeal or cornea.

12. The condition of the bronchioles and any alteration in their contraction can also be determined by recording the rate of distension or of retraction of the lung.

13. Typical bronchiolar contractions by stimulation of the vagus, can be obtained in animals recently killed by bleeding. Some constriction can even be obtained 30 minutes after death.

14. Gradual constriction of bronchioles followed by dilatation is usually seen post mortem.

15. Muscarine, pilocarpine, and physostigmine excite the vagal endings and induce typical bronchiolar constriction. The effect is abolished by atropine.

16. Barium, veratrine, bromine, and the salts of many of the heavy metals (*e.g.* gold) produce constriction which is not influenced by atropine.

17. Inhalation of CO_2 leads to constriction of the bronchioles which is not altogether central in origin.

18. Chloroform, ether, urethane, lobelia, and atropine induce dilatation of the bronchioles when constriction is present. The dilatation produced by lobelia is quite transient, whilst that by atropine is permanent.

Reprinted from the 'Transactions of the Pathological Society of London,' 1903.

The pathology of asthma.[1]

By T. G. Brodie and W. E. Dixon.

(From the Research Laboratories of the Royal Colleges of Physicians and Surgeons.)

The term "asthma" in its original meaning was used with the significance with which we now employ dyspnœa, but its use has gradually become much more restricted. It is now usually limited to the affection known as bronchial or spasmodic asthma, and in this meaning only we use the word in the present paper.

Numerous theories have been advanced to explain the cause of the acute attack, the most important being classified by Osler in the following manner:

1. That it is due to a spasm of the bronchial muscles.
2. That it is caused by a swelling of the bronchial mucous membrane—fluctionary hyperæmia (Traube), vaso-motor turgescence (Weber), diffuse hyperæmic swelling (Clark).
3. That in many cases it is a special form of inflammation of the bronchioles, *bronchiolitis exudativa* (Curschmann).
4. That it is due to a reflex spasm of the inspiratory muscles, especially the diaphragm.

Of these theories the first is the one which, at the present time, is most generally supported, but there are still many who uphold the second and third views, or modified forms of them.

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[1] Part of the expenses of this research have been defrayed by a grant from the Royal Society.

During the past three years we have studied the physiological behaviour of the bronchial muscles under many diverse conditions, and, as a general conclusion, have arrived at the view that a spasm of the bronchial muscles is in all instances the true cause underlying an acute attack of asthma. In this paper we give a brief account of the bearing which our experiments have upon the pathology of asthma, and the manner in which they can be utilised in explaining the symptoms and physical signs of an attack. Full details of the experiments up to the point at present reached are now being published in the 'Journal of Physiology.'

In most of our experiments the state of contraction of the bronchioles has been determined by recording the volume of air entering and leaving the lung during each respiratory act. This was generally effected by thoroughly exposing one lung by resection of several ribs, and then isolating one of the lobes, usually the lower lobe of the right lung, and enclosing it in an oncometer. As soon as the thorax was opened aëration was maintained by artificial respiration. This method possesses the advantage that throughout a long experiment the conditions of the supply of air to the trachea remain perfectly constant and under control. The oncometer is made air-tight by a stiff vaseline, and the changes in volume of the enclosed lobe recorded by connecting the oncometer to a bellows-recorder. The tracing obtained shows variations in the amount of blood contained in the lobe, as well as in the air waves, but the former are so much smaller than the latter that, as a rule, they introduce no difficulty in the interpretation to be placed upon the result. In most experiments the aortic blood-pressure was simultaneously recorded by a mercury manometer, thus enabling us at any time to estimate the general condition of the animal. We will, in the first place, give the results of our experiments upon the innervation of the muscles. It has previously been thoroughly established that the motor nerve to the bronchial muscles is the vagus. For a long time this conclusion was very much in question, but the experiments of MacGillavry,[1] Einthoven,[2] and Beer[3] have conclusively established it. They showed that the poor results obtained by previous observers were due to the methods they employed. The only satisfactory procedure is to prove that the movement of air into and out of the alveoli has been rendered either more difficult or more easy.

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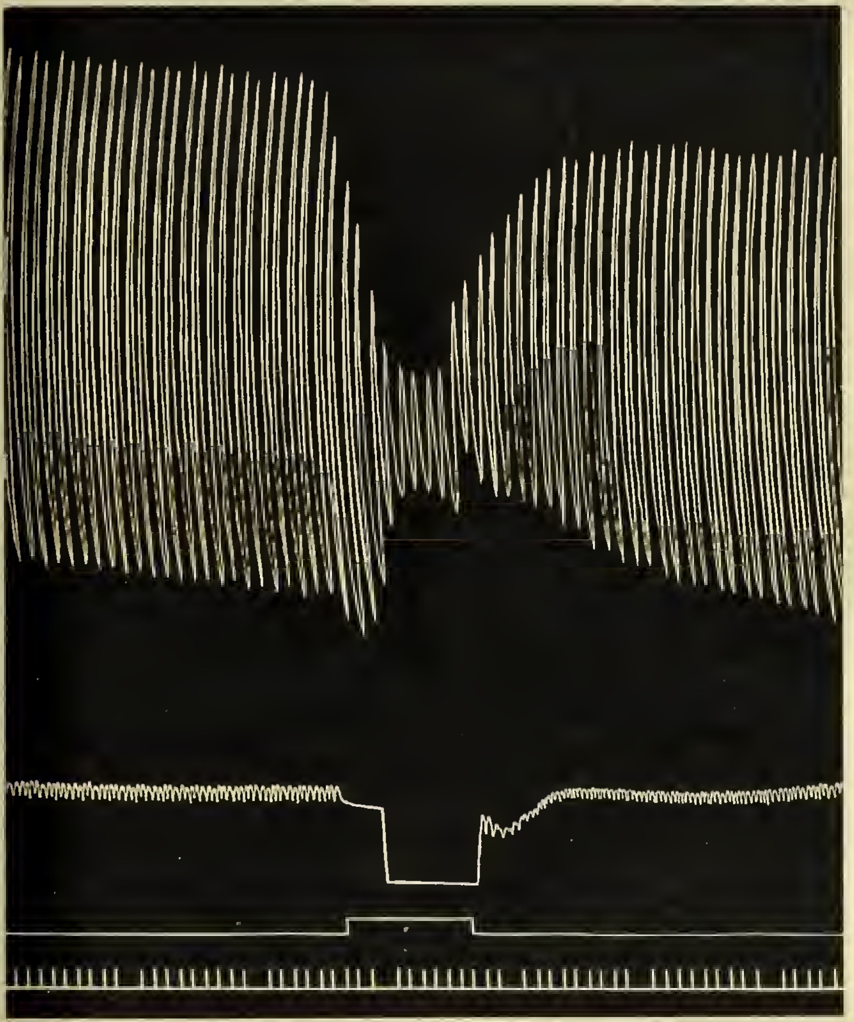
[1] MacGillavry, 'Arch. Néerlandaises des Sciences,' 1877, p. 445.

[2] Einthoven, 'Pflüger's Arch.,' li, 1892, p. 367.

[3] Beer, 'Arch. f. (Anat. u.) Physiol.,' 1892, suppl., p. 101.

FIG. 1.[1]



Dog killed by pithing. Effect of stimulation of the right vagus (coil at 5) upon the air movements of the middle lobe of the right lung.

In our experiments this is attained by recording the volume of air entering at each inflation, the rise of air-pressure in the trachea, and the total duration of that rise, being maintained quite constant for all inflations. A typical record of the result obtained is given in Fig. 1, which shows the effect of a short stimulation of the vagus upon a lobe of the right lung. The upper tracing is that of the lung-volume, and the lower the aortic-pressure curve. During the time indicated by the rise in the abscissa line the vagus was excited by induction shocks of such strength as to be just unpleasant when felt on the tongue. In the lung-volume tracing each rise represents the amount of air entering the lobe, and the fall, the amount of air leaving each time the lobe collapses. The effect of the stimulation of the vagus, therefore, is to rapidly cut down the amount of air entering and leaving the lobe. This is quickly produced, for in seven respirations the maximum decrease has been reached, and the amount of air which then entered was diminished to one fourth of the original volume; the heights of the air waves are 55 mm. before and 13 mm. after. The effect quickly passed off when the excitation was stopped. A result such as this can be repeated several times, but gradually becomes less and less pronounced, for the fibres are fairly easily fatigued to electrical stimulation. In this figure the lines passing through the apices and bases of the air waves of the lung tracing approach one another, the upper line being the one most affected. This means that the movement most restricted was that of inflation, a point we shall have to again refer to later. Similar reactions are to be obtained in the rabbit and cat, the other two animals upon which we have experimented, but in the latter animal the reaction is usually very different. The usual effect in the cat is a slight transient constriction, followed by a dilatation lasting until the excitation ceases, and about forty seconds later followed by a typical constriction. This reaction led us to conjecture that we were dealing with a twofold effect, one part tending to produce constriction, the other relaxation. We therefore proceeded to test for broncho-dilator fibres in the vagus, and soon succeeded in proving their presence. We had already shown that there is no tonus present in these muscles under normal conditions, and as, before it could be possible to show the action of such fibres, the muscles must be contracted, we, in the first place, induced a tonic contraction. This can be readily effected by the administration of muscarine or pilocarpine. Excitation of the vagus now produces dilatation of the bronchioles, an effect well seen in Fig. 2. In this experiment the air movements had been cut out completely by a dose of pilocarpine (3 mgrms.), and on now exciting the vagus the bronchioles relax and allow a considerable volume of air to enter and leave the lung with each respiratory act. Shortly after the excitation ceases the bronchioles again constrict under the influence of the drug. This reaction may be repeated a great number of times with precisely the same result on each occasion. Similar effects were also obtained in the dog and rabbit,

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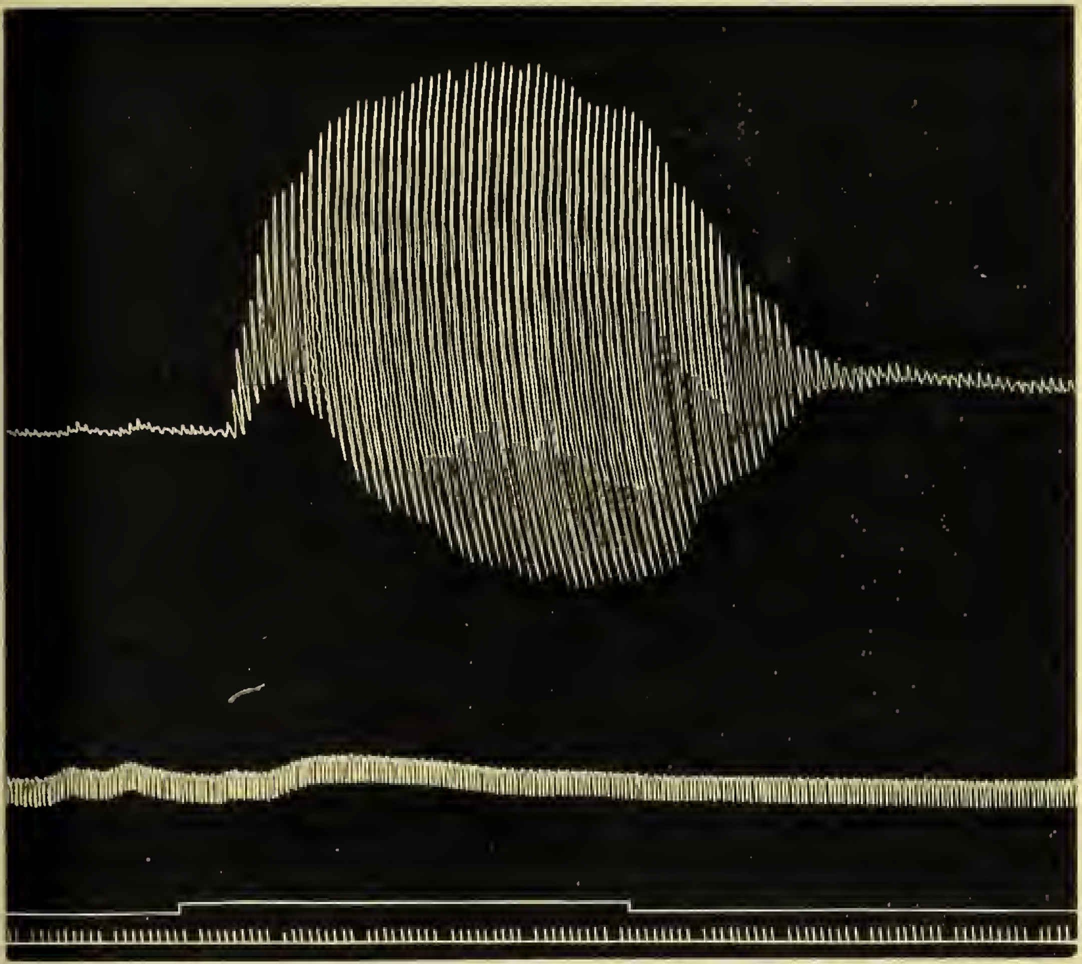
[1] All the figures in this paper are to be read from left to right. The time record in all indicates seconds. The blood-pressure was recorded by a mercury manometer connected to the carotid. In the lung plethysmogram, a rise of the lever indicates increase in volume of the lobe.

Fig. 2.



Cat, killed by pithing. Effect of excitation of the vagus (coil at 4 cm.), upon a lung in which the bronchioles had been constricted by a dose of pilocarpine. Figure reduced to two thirds.

though apparently the dilator fibres are not quite so well developed in these animals as in the cat.

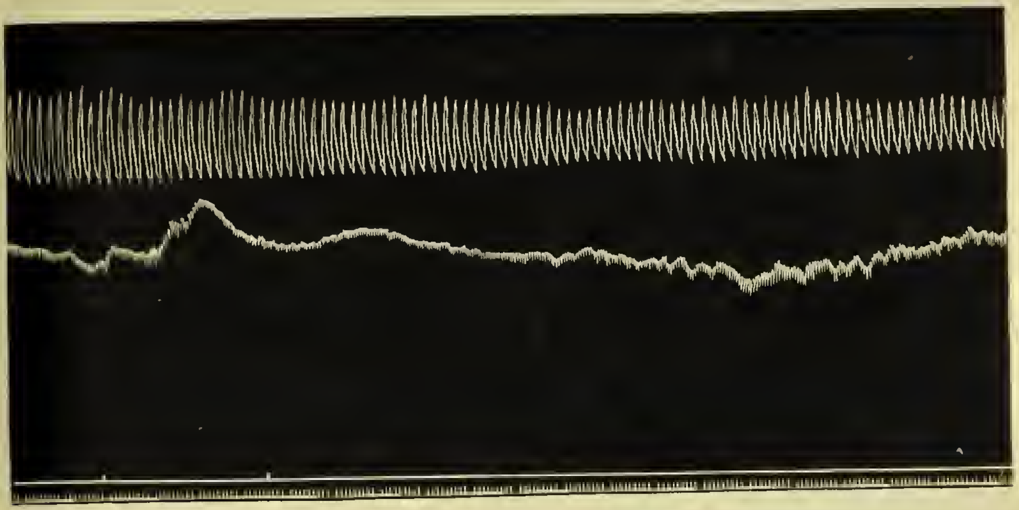
The bronchial muscles are therefore innervated by two sets of fibres, a constrictor and a dilator set, both of which run in the vagus. An examination of the sympathetic fibres leaving the upper thoracic cord for motor fibres to these muscles gave completely negative results.

A considerable difficulty we met in all our early experiments, and which we only learnt to explain after much trouble, was due to the action of the anæsthetic. A large number of our early experiments gave negative results, which we ultimately found to be caused by the use of chloroform or ether as the anæsthetic. These, we found, paralysed the nerve-endings in the bronchial muscles, and we only succeeded in obtaining constant results on vagal stimulation when their use was reduced to a minimum. We therefore adopted the practice of anæsthetising the animal with the smallest necessary quantity of chloroform, and then destroying the medulla and brain. No further anæsthetic was then required.

It is necessary to mention this here because it explains the great difficulty we have experienced in obtaining reflex effects. Many observers have attempted to get reflex constriction of the bronchial muscles, but have only succeeded in a very partial degree. Thus, slight reflex constrictions have been described as following excitation of the sciatic, superior laryngeal, central end of the vagus, the cornea, and the nasal mucous membrane, but the tracings given in support of these statements are far from satisfactory. When working upon anæsthetised animals our attempts to produce these reflexes were almost entirely negative. It therefore became necessary to avoid the use of an anæsthetic altogether, or only to administer it for a very short time. As it was also necessary to keep the medulla uninjured, we worked upon decerebrate animals, but the conditions were still far from ideal, for the decerebration involves a rather lengthy operation under anæsthesia, and the injury produced in the majority of cases appears to set up a constant inhibitory effect upon the medullary centres. When working in this way, we therefore decerebrated the animal and then kept it for some three or four hours before proceeding with the experiment, in order to allow ample time for recovery from the anæsthetic. But even under these conditions the results obtained have not proved as successful as we had hoped, and while giving the reflex effects we have at present succeeded in recording, we feel that there are still many undecided points to be worked out before we can understand the normal working of these centres. This is all the more unfortunate as the reflex mechanism unquestionably plays a most important part in the production of the acute asthmatic attack.

We have never succeeded in getting reflex constriction by excitation of a sensory cutaneous nerve, nor by excitation of the stomach or intestines, but we have at times recorded slight bronchial constriction on excitation of the central end of the vagus divided in the neck, or of the superior laryngeal. We never obtained any result from exciting the cornea. The most important reflex is, however, from the nasal mucous membrane. In all cases electrical, mechanical, or chemical excitation produces some effect. One of the best results is given in Fig. 3, in which the constriction produced results in a diminution of the volume of air entering the lung to one half its original volume. The tracing also illustrates well one other point, viz. the gradual onset of the constriction and its very prolonged course. Section of the vagus entirely abolishes the result, and

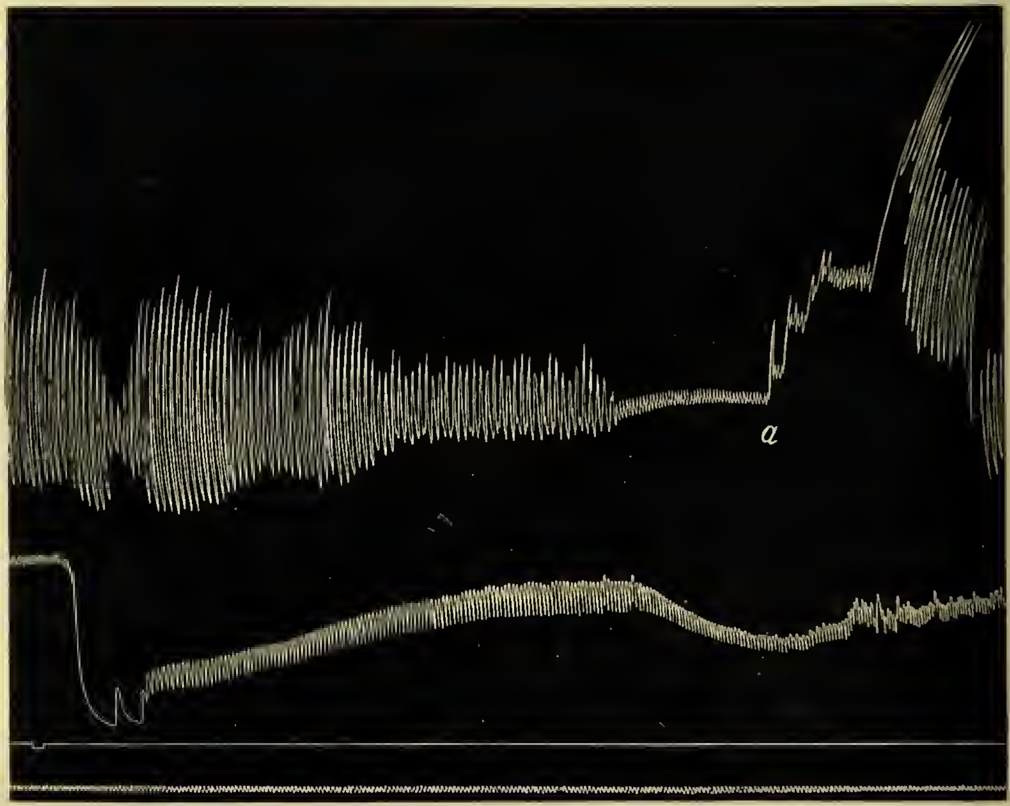
FIG. 3.



Dog. Decerebrate. Right lung-volume. Blood-pressure. Shows the effect of stimulating a point well back in the left nostril. Figure reduced to one third.

if the vagus be divided after the reflex constriction has been set up, the bronchioles at once relax. We have further observed that the upper and posterior part of the nasal septum is the best area from which to produce the reflex. From the experience gained from experiments of this class we feel quite sure that vigorous reflex effects are to be obtained, if we can find a method by which the centre can be isolated in a fairly normal state.

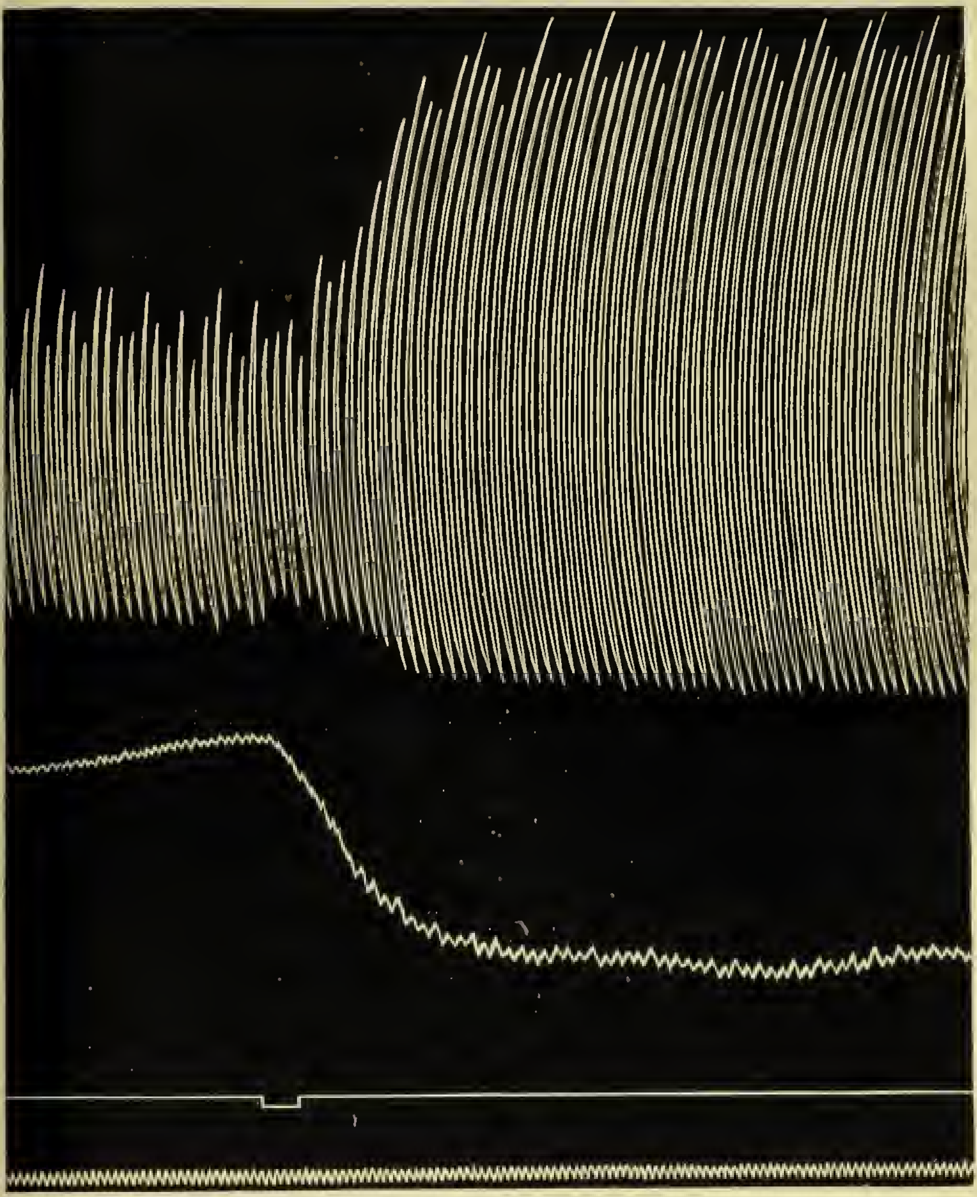
Fig. 4.



Cat. A.C.E. Injection of 0·001 grm. of muscarine sulphate. At the point marked "*a*" the force of inflation was increased. Figure reduced to one half.

We next come to the action of drugs upon these muscles. Muscarine, pilocarpine, physostigmine, and digitalin produce marked constriction by peripheral stimulation of the vagal endings. Gold salts, barium salts, and veratrine produce constriction by a direct action upon the muscle-fibres. Atropine, hyoscyamine, and hyoscine paralyse the vagal endings, and thus lead to dilatation if constriction is present. As examples of these effects we give Figs. 4 and 5. Fig. 4 shows the result of injecting 1 mgrm. of muscarine, which is seen to produce a complete closure of the bronchioles to the ordinary pressure of inflation. It is also to be noted that slight over-distension of

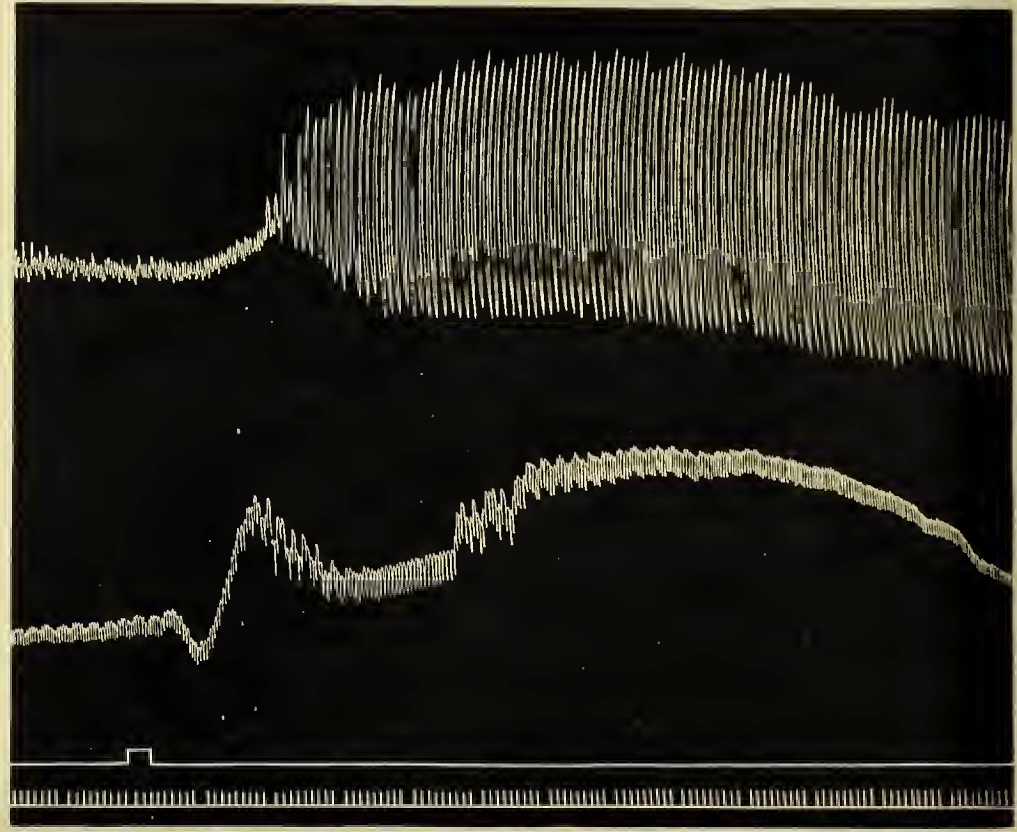
Fig. 5.



Cat. Lung-volume. Blood-pressure. Some bronchial tonus had previously been set up by an injection of pilocarpine nitrate. Shows the effect on the bronchioles of an injection of atropine sulphate into the jugular vein.

the lobe is produced, a small quantity of air being permanently imprisoned in the lung. At the point marked "*a*" the force used to inflate the lungs was increased, and at once marked over-distension was produced, a point to which we return again presently. Fig. 5 shows the effect of administering atropine while the bronchioles are constricted by a previous dose of muscarine. Dilatation is at once produced.

Fig. 6.



Cat. A.C.E. Lung-volume. Blood-pressure. Shows the effect of an injection of the B.P. tincture of lobelia into the jugular vein. The animal had previously been given a small dose of pilocarpine, and the bronchioles were so constricted at the time of the tracing that no air was entering or leaving the lung. The zero of blood-pressure is 75 mm. below the abscissa line. Figure reduced three quarters.

Lobelia produces a most interesting result, especially of importance because of the place the drug occupies as a specific in the treatment of asthma. Fig. 6 shows the result of an intra-venous injection of the drug in an animal in which bronchial constriction had been produced by pilocarpine. It quickly produces relaxation, but, unlike the similar relaxation of atropine, the effect is quite transitory, for in a short time the contraction returns, though it is not shown in this figure. Repeated injections of the drug can produce a series of such transitory effects, which in many particulars recall the broncho-dilatation produced by exciting the vagus in such animals.

Morphine also tends to produce relaxation when tonic constriction is present, but the dose required is relatively high, and the relaxation is never quite so marked as that following atropine or lobelia.

The action of carbonic acid is of very great importance. It acts chiefly, directly upon the medullary centre, producing a marked tonus, which may amount to a restriction of the air movements to one half their previous extent. In some instances we have also found the gas to exert a peripheral effect upon the nerves, for the tonus set up was not abolished, in all cases, by section of the vagus in the neck, although it was cut out by atropine.

We now come to the consideration of the bearing of these experiments upon asthma, and as a large number of our experiments have been concerned with the bronchial muscles, our main object will be to examine how far the theory of bronchial spasm can explain all the symptoms of asthma. The general history of an attack of asthma, its suddenness of onset, and rapid final recovery, indicate a nervous origin, and point to an excessive action of some physiological process carried to an abnormal degree. Practically all observers are agreed that the essential cause of the attack lies in a diminution of the lumen of the bronchioles, for the theory of diaphragmatic spasm has been shown to have been founded upon an erroneous interpretation of the symptoms and physical signs of an attack, and of the experimental data which appeared to support it. We need not further discuss it, and our issues, therefore, become narrowed to the three possible causes which can produce a diminution of the lumen of the bronchioles, viz. contraction of the bronchial muscles, acute congestion of the bronchial mucous membrane, or partial plugging of the bronchioles by secretion. A considerable proportion of cases of asthma are unattended by any expectoration, and the physical signs observed during an acute attack are much more those due to a restricted, but clear, lumen than to tubules containing a quantity of accumulated secretion.

The histories of asthmatic patients clearly indicate that the bronchitis is really secondary, a complication introduced by the highly abnormal state set up by the repeated attacks. For these and other reasons, to which we return presently, we think that the suggestion that the attack is due to accumulated secretion may be dismissed. We must therefore turn our attention to the other two possible causes, vascular engorgement and spasmodic muscular contraction. It has been argued that an acute vascular engorgement, quite similar to that which is well known to occur in the nasal mucous membrane, may affect the mucous membrane of the bronchioles, but we must point out that this is extremely unlikely, for the structure of the two membranes is most dissimilar. The nasal mucous membrane, especially over the inferior turbinate bone and lower nasal passages, is extremely vascular, and in many parts large venous plexuses are found encircled by bundles of muscular fibres, thus forming a sort of cavernous erectile tissue (Klein). The bronchial mucous membrane, on the other hand, is thin, and possesses what is, in comparison, a relatively insignificant blood-supply.

Of the points in favour of accepting the view that asthma is essentially a contraction of the bronchial muscles, the first undoubtedly is that the sudden onset and rapid disappearance of the attack exactly correspond with what we know to be the common course of a prolonged contraction of involuntary muscle. Again, a perfectly typical attack of asthma can be induced in an animal by giving it a small dose of muscarine. The dyspnœa and extended thorax are typically produced, and auscultation reveals the sibilant râles so characteristic of the disease. Moreover our experiments have proved that the whole of these lung symptoms in animals are due to the bronchial spasm. Practically no engorgement of the lungs is produced, and if only small doses of the drug are employed there is no excessive secretion into the bronchi and bronchioles. Moreover the attack thus induced may be instantaneously cut short by an injection of atropine, the recovery being complete, as is well seen from Fig. 5. In this instance it is impossible that any accumulation of secretion, or the presence of an œdema, could possibly have been the cause of the respiratory symptoms, as was at one time suggested by Grossmann. And, lastly, the conclusion is finally settled by the fact that excitation of the vagus in these animals at once leads to recovery without modifying the heart or vascular system.

A further very strong argument in favour of this spasm theory is to be drawn from the study of the action of those drugs which have been empirically discovered to be of value in asthma. Foremost amongst these we may place chloroform and ether. We have already pointed out that it is almost impossible to produce a contraction of the bronchial muscles by stimulating the vagus in animals anæsthetised by either of these bodies. They act by paralysing the nerve-endings, which are exposed to concentrated doses of the vapour directly after its absorption through the mucous membrane. Just as stopping the anæsthetic will permit the production of a contraction by nerve excitation, so it is the common experience that if the dose of anæsthetic inhaled be too much reduced the symptoms again appear, although a remission from the attack had been gained by its use. We may consequently argue that it is probable that the attack is cut short because the drug has paralysed the vagal endings, which would imply that the constriction was originally produced by some excitation of those endings or by impulses passing down the vagi. That ether, especially, acts favourably in relieving the acute attack is distinctly against the view that the attack is due to a vascular change, for ether, as is well known, produces both hyperæmia of a mucous membrane and increased secretion from its glands, and should therefore tend, on this hypothesis, to make matters worse rather than better.

The next series of drugs we may consider are the alkaloids of the atropine series. All act similarly upon the bronchial muscles, producing complete relaxation when tonus is present, and all have attained high repute in the treatment of asthma. Atropine, in doses sufficient to completely paralyse the vagal endings, lowers the blood-pressure and dilates peripheral vessels. It thus tends to produce some engorgement of the mucous membrane, but, on the other hand, it would stop secretion. Its beneficial action in an acute attack, therefore, could only be explained on the vascular theory if there were some muscular mechanism in the bronchial mucous membrane which could be paralysed by atropine, and which when in activity produced marked congestion of the membrane. We have pointed out that no such mechanism is known to exist.

Morphine is another drug which has proved of the highest value in asthma, and, again, morphine we found to produce relaxation if tonus were present; but altogether apart from this action, morphine exerts a sedative action on all nervous centres, and this in itself could explain the beneficial action of the drug.

One further drug which it is necessary to consider is lobelia. The ethereal tincture of lobelia, when diluted, freed from ether, and then injected into an animal in which bronchial spasm has been produced by muscarine or pilocarpine, produces a most characteristic effect, viz. temporary relaxation (see Fig. 6, p. 26). The benefit only lasts a short time, but may be reproduced indefinitely by injecting further doses.

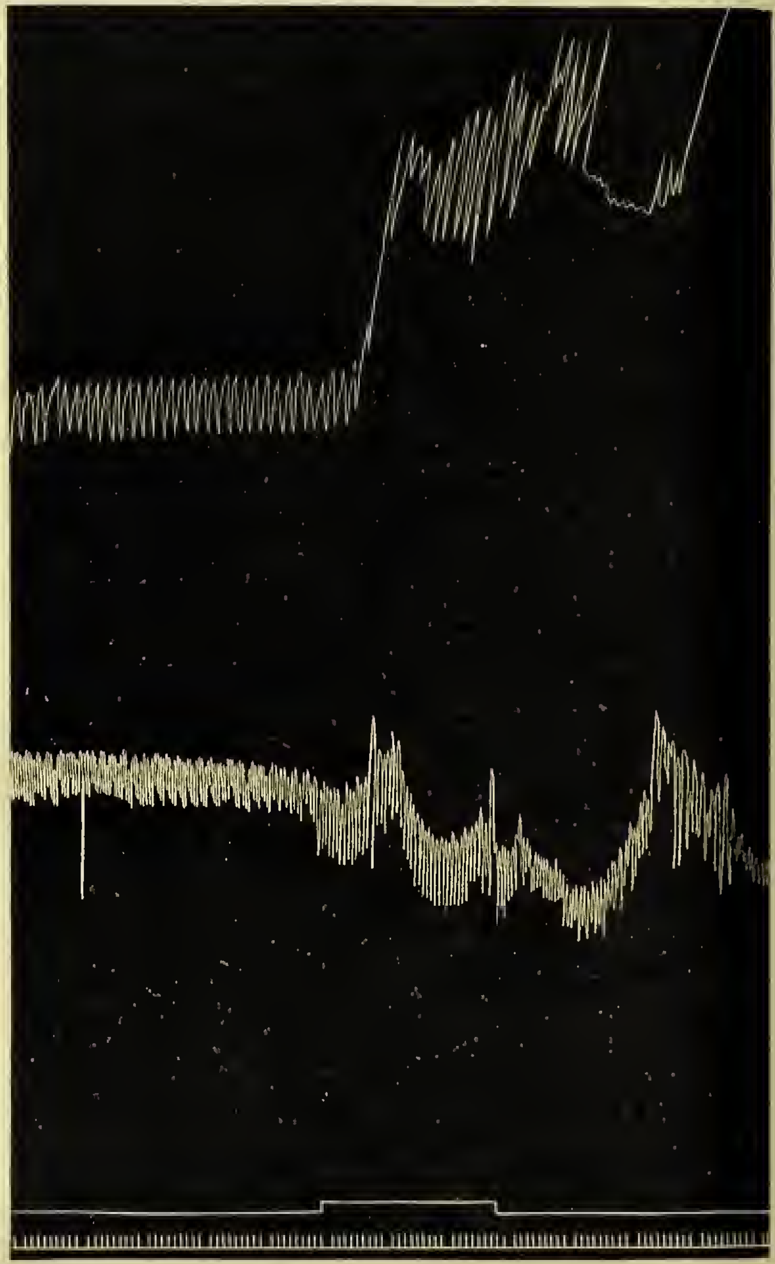
In the last place, there is one other drug which has been warmly recommended by several physicians as useful in asthma, but which we should at first sight have unhesitatingly condemned in strong terms, viz. pilocarpine. Pilocarpine in moderate doses produces typical bronchial constriction, but when testing the action of small doses we found that they produced no constriction. When experimenting with muscarine, a drug which in most of its physiological characteristics closely resembles pilocarpine, we frequently noted that it first produced relaxation before the constriction. In a few experiments we studied the action of minute doses of pilocarpine upon a lung in which the bronchioles were constricted by stimulating the vagus, and found that increased air movements into the lobe experimented upon resulted. This probably means dilatation of the bronchioles, but we cannot decisively state it to be this at present, because there is one other possible explanation, viz. that the increased expansion was due to bronchial constriction produced in the opposite lung. There is yet another possible explanation of any beneficial action of pilocarpine in asthma, viz. that the secretion copiously produced from the mucous surfaces by its use may for a time check or allay an abnormal process which had been operative from any of those surfaces.

No theory of asthma can be complete until it can adequately explain the whole of the symptoms of the acute attack. Perhaps the most important of all is the over-distension of the lung, which is so universal a symptom, and which has been the cause of the rejection by very many observers of the view we are now upholding. In the typical tracing we gave in Fig. 1

no over-distension is produced, but that is entirely a question of the procedure adopted for the experiment. If it is wished, one can in every experiment produce this over-distension by slightly altering the mode of artificial respiration; but we have not done so in our experiments because, though the production of an over-distension is the most striking feature of a tracing taken during the production of marked constriction, yet the method is not so sensitive for the purpose of showing small changes in the calibre of the bronchioles. We will therefore consider how the over-distension is to be produced in the first place in an exposed lung inflated rhythmically by an artificial respiration apparatus; and secondly, in a lung still within the intact thorax. We can then determine whether such results concur with the observed facts of the respiration and over-distension of the thorax in an asthmatic attack.

Over-distension occurring with bronchial constriction can be produced in the exposed lung by altering either of two factors in the artificial respiration, viz. (1) the force of inflation, or (2) the time allowed for deflation. Thus Fig. 7 illustrates the production of over-distension on excitation of the vagus. The effect was produced in this instance by shortening the time allowed for the lobe to empty itself in deflation. In the next place, if we refer again to Fig. 4 we see that at the point marked "*a*" in that figure over-distension is produced. This was caused by an increase in the pressure of the distending air, produced by partially closing the lateral orifice in the tracheal cannula, a change which became necessary because the animal was receiving no air into its lungs and was rapidly becoming asphyxiated. The explanation of the production of the over-distension in either of these two instances is not far to seek. The force producing inflation is relatively great (some 100 to 120 mm. of water at its maximum), but lasts only a very short time. The force emptying the lung, *i. e.* producing deflation, is relatively weak, for it is the elastic recoil of the distended alveoli and pulmonary tissue generally. It also becomes less the smaller the alveoli become, but, on the other hand, it is acting, as a rule, for a long time. Suppose, now, without in any way altering the force employed for inflation or its duration, we gradually decrease the time allowed for deflation, a stage is presently reached in which the recoil of the lung is not sufficiently powerful to enable it to drive out as much air as had been driven in during inflation. In other words, the lung begins to permanently over-distend—a condition which can be produced in any lung, no matter what the state of contraction

Fig. 7.



Cat. A.C.E. Right lobe in oncometer. Excitation of the right vagus by weak induction shocks (coil at 8 cm.) produces marked over-distension of the lobe.

of the bronchioles may be. Now suppose that in a lung in which this stage has just been reached the bronchioles are made to constrict. This introduces a further resistance to the air movements, which affects both inflation and deflation. But inflation is much more powerful than deflation; hence it will affect inflation less—a point we have abundantly proved by recording the rate of filling and emptying respectively. As a result, then, the lung is unable to empty as quickly as it is filled, and over-distension results, because no reserve of time is allowed for deflation.

A somewhat similar argument explains the production of over-distension when the distending force is increased. This results in greater filling of the alveoli, but the maximum alveolar pressure does not rise in proportion. Consequently, though the force of deflation is higher, it yet takes a longer time to empty the alveoli than before, and should the condition be carried a little farther it may not have had time to empty completely, and over-distension results. As a rule, with an ordinary respiration pump and a normal lung this stage can never be reached, because sufficient time is always allowed by the mechanism for the lung to empty itself. In the course of our experiments we have, however, met with two animals which had at some previous time suffered from pleurisy, and part of the lung had become adherent to the thoracic wall. After a good deal of care we succeeded in separating one of the emphysematous lobes, and found in both instances, as, indeed, we quite expected, that over-distension was most readily producible. Fig. 7 is taken from the records yielded by one of these animals, and others are given in our paper in the 'Journal of Physiology.' While, therefore, it is not possible to produce any really marked degree of over-distension in a normal lung by an increase of the distending pressure within approximately normal limits, it at once occurs if the bronchioles be made to constrict. Again, the explanation is as before, viz. that the resistance is increased, both to inflation and deflation, but the diminution in movement of air is most marked in deflation, although some effect is produced on the inflation. The effect, moreover, is only seen well when the constriction is great.

The production of over-distension in the lungs while within the thorax is to be explained on exactly analogous lines.

Under normal conditions the force of inspiration is much greater than that of expiration, and acts to better advantage. By violent respiratory movements it is possible to increase the force both of inspiration and expiration very greatly. But the mechanics of the thorax permits a more marked increase of the power of inspiration than that of expiration :—the thorax is an inspiratory mechanism rather than an expiratory. Consequently, with normal respiration, marked constriction of the bronchioles is followed by over-distension. But in asthma, and in the asthmatic attack produced by muscarine, there is a yet more vital point to be observed. This is, that in both instances the effort is inspiratory. The patient exhibits violent inspiratory movements, whilst expiration is quiet and prolonged. In text-books of medicine sufficient stress is not laid upon this ; indeed, many have described the type of respiration present during an attack of asthma as being characterised by increased force of the expiratory movements, and in nearly all instances the description is that the *respiration* is violent, not as it should be, that the *inspiration* is violent. The whole endeavour on the part of the patient is to get air in, not to drive it out. This was first clearly pointed out by Einthoven, who also showed that, though the condition leads to marked over-distension, yet, under the circumstances, it is the best possible thing for the patient, and chiefly because, if the force of expiration should have become excessive, some of that force will be transmitted to the outer surface of the already constricted bronchioles, and tend to make them collapse still further.

If asthma is due to contraction of the bronchial muscles, we must attempt to decide how that contraction is produced. Is it peripheral, central, or reflex? If peripheral, it might be due to a direct action upon the muscle-fibre or upon the nerve-fibre. As the attack generally involves the whole of both lungs, we could only assume that it was due to some chemical irritant uniformly distributed by the blood. The fact that atropine, chloroform, morphia, lobelia, etc., are all efficacious in relieving the attack tends to show that the action is *viâ* the nerves. The probability is that it is of central or reflex origin; but the other view, viz. that it might be of peripheral nervous origin, must also be considered. Mr. S. G. Shattock made the suggestion to us that possibly there was a local chemical irritant, and that in that case it might be found in the sputum of an asthmatic patient. With his collaboration, we therefore performed a few experiments in which saline extracts from asthma sputum were injected intra-venously and sprayed into the air-passages. The results were completely negative. We also found that a solution of Charcot-Leyden crystals intra-venously injected was quite harmless.

We are not in a position to decide against the view of a peripheral cause in all attacks, but the facts that the whole of both lungs are usually involved, that the onset is sudden, and that only this particular set of muscle-fibres are involved, indicate a central origin, a supposition generally accepted by all, and strongly confirmed in that the patients in whom it is usually found are almost universally of the nervous type. If the attack is produced from the centre the acting cause may directly attack the centre itself, or a peripheral surface or organ. In this connection it is very important to note that carbonic acid in excess in the blood causes considerable activity of the centre in the medulla, probably by a direct action upon it. At any rate, the effect is not peripheral in most cases, for usually it is abolished by section of the vagi. The importance of this lies in that, in the paroxysm of an attack, carbonic acid is accumulated in the blood, and must therefore tend to still further increase and maintain the abnormal activity of the centre.

It is not probable that the cause of the attack is some direct irritation of the centre, otherwise we should expect to find some gross lesion of the centre, probably also involving neighbouring centres; and even if the exciting cause were a chemical irritant, analogy would lead us to expect some action upon other nerve centres. The probability therefore is, that the effect is produced reflexly, and it is in this direction that supporters of the muscular-spasm theory of asthma have sought for explanations of the disease. From the clinical side there is much evidence tending to bear this out. Thus in asthmatics it is most important to maintain the digestive tract in a normal state. An attack can with certainty be produced, in some patients, by an injudicious meal. Some have suggested that the sight alone of certain animals or objects can induce an attack, and there does not seem to be any question that the smell of certain animals (*e. g.* the cat) can produce an attack in some cases. With regard to the respiratory tract, there are fair grounds for believing that in the predisposed an abnormal state of the bronchiolar, bronchial, tracheal, or laryngeal mucous membrane may induce, or at any rate favour, the onset of an attack. But the mucous membrane, which has most generally been acknowledged to play the most important part in asthma, is the nasal.

As already described, we have studied the reflex activities of this centre in a large number of experiments; but not with the success we had hoped. Still, our experience encourages us to persevere, for we can prove that the reflex excitability has always been considerably diminished in our experiments, so that those reflexes we have succeeded in obtaining must of necessity be much more active in a normal intact animal. The one reflex which has given us good results has been that from the nasal mucous membrane, and it has always shown the distinctive character of prolonged duration. Chemical, mechanical, and thermal irritants have all been effective. All this coincides well with very many facts that are known as to the causes predisposing to an attack of asthma. Odours, or minute changes in the atmosphere, which are perfectly imperceptible to the normal individual, can excite an attack in the asthmatic. Hay fever and asthma are well known to be closely connected in some causal relationship. Many complete cures of asthma have been effected by operative treatment of the nose. Dr. Greville MacDonald informs us that he finds those cases are the most favourable for operation in which sneezing is a prominent symptom, and that he has obtained the greatest success in cases in which there have been small growths on the inferior turbinate, which could be removed. The results recently recorded by Francis are so striking that they deserve the most attentive study. He states that nearly all cases of asthma may be permanently cured by cauterisation of a small area of the upper and posterior portion of the nasal septum. He expressly states that in many of his successful cases nothing abnormal was to be seen in the area cauterised. If, as Francis believes, the originating cause in practically all instances is some abnormal state of a part of the nasal mucous membrane, it would then be necessary to explain how unusual stimuli, arising frmo the stomach or lungs, could favour or even induce an attack. It may well be that the abnormal state of the nasal mucous membrane sets up such a degree of hyper-excitability of the centre that any further stimulus, which under more normal conditions would excite no activity in the centre, can now suffice to produce the violent discharge originating the attack.

On the view that bronchial spasm is the one and only cause of asthma, the bronchitis always found in chronic asthmatics is the direct consequence of the abnormal states to which the lungs are subjected in the acute attacks. Many cases of asthma are known in which there is no bronchitis; indeed, the rule is that the latter develops after the asthma has obtained its hold. On the other hand, asthma is comparatively rare in cases of ordinary bronchitis, and the symptoms observed in the latter disease, even when there is but little secretion, are totally different from those of asthma, although we know that the mucous membrane is inflamed. In our opinion, as we have already stated, it is most unsafe to argue that, because the nasal mucous membrane can rapidly become turgid and swollen, and as rapidly recover, a similar thing can occur in the bronchi. Thus, in Clifford Allbutt's 'System of Medicine,' Goodhart writes: "It seems, therefore, a rational belief that what can be proved to exist in the upper air-passages—namely, a definite erection or turgidity of the nasal mucous membrane—may also take place lower down in the bronchial mucous surface, and, so doing, accomplish much of what we call spasmodic asthma." The onus lies with those who support this view to prove that there is such a vascular mechanism in the bronchial mucous surface which can permit of the production of such turgidity; the statements of histologists are directly opposed to it. We have in several experiments aimed at getting maximum turgidity of the mucous membrane by ligaturing or clamping the pulmonary vein issuing from a lobe, but we have never observed anything in the least comparable to the restricted air movements observed when the bronchioles are made to contract.

Our general conclusions, therefore, are that true bronchial asthma is in all cases a spasmodic contraction of the bronchial muscles; that if it follows or occurs in an acute attack of bronchitis, it has been reflexly produced either from the bronchial mucous membrane or from some other hyper-irritable mucous membrane, or possibly from the combined action of the two abnormal surfaces acting on the medullary centre. The over-distension of the lung is the result of the bronchial spasm, and is produced because the action of filling the alveoli is always effected at a greater advantage than that of emptying, and that if extraordinary efforts at expiration are made, they do but serve to further impede the emptying of the distended lung. The state of the thorax and the modified respiratory movements are the direct consequence of the over-distension and the spasm. The increased violence of inspiration is due to the great force required to get air through the constricted bronchioles, and the so-called inspiratory spasm is the evidence of the over-distension. The chest does not collapse, and therefore the respiratory muscles and ribs cannot fall into the true expiratory position, in which movements of small extent result in considerable increase in capacity of the thorax. The violent movements observed consequently only result in a relatively small increase in capacity, and this in turn does not result in so large a fall in the alveolar pressure as would be produced by a similar volume increase in a less distended set of alveoli, because at the start the alveoli are well distended and filled with air practically at atmospheric pressure.

Having seen that reflex contraction of the alveoli can be produced, it necessarily follows that we should inquire—what is their significance, and what important function do they normally perform which has called them into existence? We have seen that the nasal reflex is the important one, and in considering its normal significance a study of the other reflexes obtainable from this surface is most important. The reflex effects which may be produced from the nasal mucous membrane are cardiac inhibition, dilatation of peripheral blood-vessels, arrest of respiration in the expiratory phase, spasmodic closure of the glottis, coughing, sneezing, and contraction of the bronchial muscles. All these are most easily produced, and their obvious meaning is to prevent the entrance of irritating substances, both particulate and molecular, into the respiratory tract. They can all be produced by chemical or mechanical excitation, and by blowing finely divided particles into the nostrils. Unquestionably one of the most effective results for the end in view is the contraction of the bronchial muscles, and this, therefore, appears to be the teleological meaning of the reflex in question. This, too, fully explains the excessive sensitiveness of a large number of asthmatic patients to minute changes in the character of the air breathed, even though those changes are quite unappreciated by normal persons. *December 16th*, 1902.

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THE CHANGES IN LENGTH OF STRIATED MUSCLE UNDER VARYING LOADS BROUGHT ABOUT BY THE INFLUENCE OF HEAT.

By T. G. BRODIE, M.D., *Lecturer on Physiology*, AND S. W. F. RICHARDSON, M.B., F.R.C.S., *Demonstrator of Physiology, St Thomas's Hospital Medical School.* (Thirty-eight Figures in Text.)

(*From the Physiological Laboratory, St Thomas's Hospital Medical School.*)

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- V. Some experiments upon the changes in elasticity and extensibility of india-rubber caused by a rise of temperature.
- VI. Criticism and conclusions.

I. HISTORICAL.

THE results of previous workers upon this subject have not been very accordant. Schmulewitsch[1] describes two effects produced by heat acting on a muscle: (1) a physical effect produced as the temperature rises from 2°—28° C., (2) a physiological effect produced at a higher temperature. He states that a muscle contracts when warmed up to 28° C., and elongates when afterwards cooled. This effect he only obtained on irritable muscle, but the reverse effect was obtained when the irritability was lost. He describes that on keeping a muscle for some time at 28° C. at first a contraction, followed in about 4 minutes by an elongation, was produced, the muscle remaining irritable the whole time. At 35° C. a second contraction of shorter duration than the preceding one was found, the muscle still remaining irritable. At 40° or 41° C. the muscle entered into heat rigidity and then its irritability was lost. The method he used was to immerse a gastrocnemius preparation in "normal saline" solution, which was then gradually warmed. In all his experiments the load was the same, viz. about 5 grams.

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[1] Schmulewitsch. *Comp. Rend.* LXVIII. p. 936. 1869.

Samkowy[1] also found that warming a sartorius from 2°—32° C. caused shortening, which disappeared on again cooling if the muscle were still living. He was not able to obtain this result in dead muscle. In his experiments he used Gruenhagen's thermotonometer[2]. Here again the load was not varied but was always low, all parts of the apparatus being made as light as possible.

Gotschlich[3] in a very complete piece of work upon the subject divides the effect of heat into two parts. He found that in some cases heating from 30° to 32° C. caused contraction, which was followed by relaxation on cooling. This result was very inconstant, was quite independent of the presence or absence of irritability and could be frequently repeated, though not after heat-rigor had been produced. In contradistinction to Schmulewitsch and Samkowy he was never able to observe any contraction by exposing a muscle to 27° or 28° C., even for over half-an-hour. In all his cases he experimented upon a sartorius, in a moist air chamber under a tension of 2 to 3 grams. At a higher temperature, 35° to 40° C., heat-contraction sets in. He describes a condition of partial heat-contraction brought about by applying the heat for a shorter time, until only a part of the heat-contraction had been produced. The muscle will slowly relax from this contraction if the temperature be lowered, and can be made to recontract if warmed a second time. In this state the extensibility of the muscle is considerably diminished.

II. THE METHOD EMPLOYED.

In all our experiments we have limited ourselves to either a sartorius preparation or a conjoint semimembranous and gracilis. These muscles were selected because their fibres run longitudinally and, practically, the whole length of the muscle. In this respect the sartorius is the more perfect of the two, and we have always employed this when practicable. The latter has the further very great advantage of being thin, so that where its temperature is rising or falling, due to a change in the temperature of the surrounding medium, it is more likely to have the same temperature throughout its whole thickness than would be the case with a larger muscle. It has the disadvantage that it is difficult to obtain satisfactory fixing points at the two extremities for those experiments where we wished to employ high loads.

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[1] Samkowy. *Pflüger's Archiv* IX. 399. 1874.

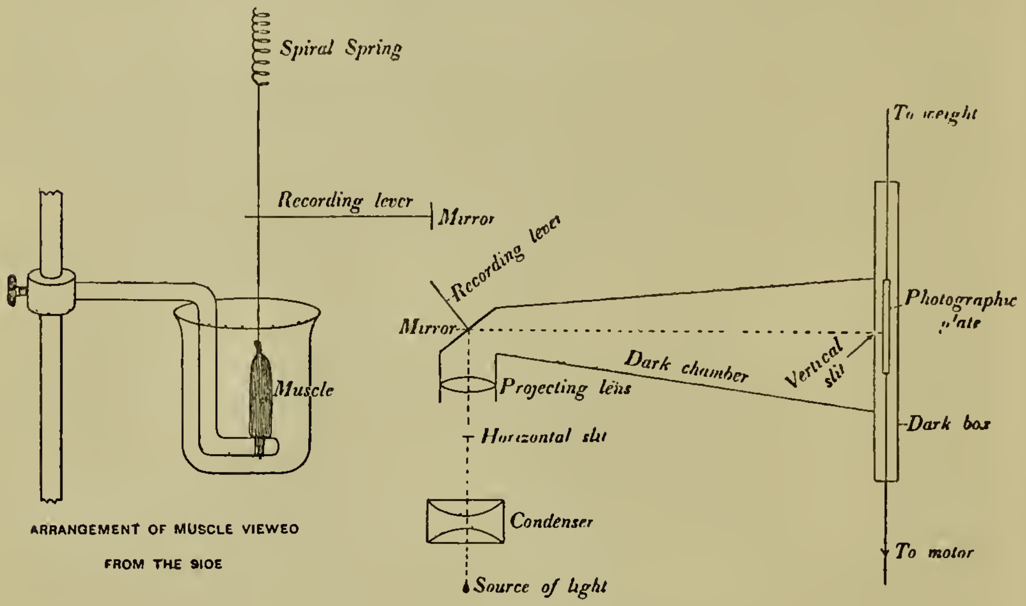
[2] Gruenhagen. *Pflüger's Archiv* XXXIII, p. 59. 1884.

[3] Gotschlich. *Pflüger's Archiv* LIV. p. 109. 1893.

The method of heating was to immerse the muscle in a fluid medium. The alternative method of suspending the muscle in a moist air-chamber did not seem to us to offer the possibility of enabling accurate readings of the temperature of the muscle. The medium we at first chose was "normal saline" solution, but owing to the known injurious effect of that solution on frog's muscle we soon came to the conclusion that it would be wiser to employ some fluid of perfectly neutral qualities. Of such fluids one might choose such as are not miscible with water and which would not diffuse into the muscle substance, such as mercury or oil, or we might adopt Locke's gum solution, or serum, or blood. Ultimately we chose defibrinated ox-blood diluted with four times its volume of normal saline. We found that fresh muscles might be kept in this fluid for prolonged intervals without in any way losing their irritability or power of contraction. The only effect we have noticed is a staining with hæmoglobin if muscles are kept in the fluid for very long times. It seemed to us that many of the contradictory results obtained by previous workers might probably be due to the fact that some worked with muscles immersed in salt solution, others with muscles in air.

The record of the alteration in length was obtained by a photographic method. The general arrangement of the apparatus is shown in the accompanying diagram (Fig. 1). The muscle under experimentation is firmly fixed at its lower end to the horizontal limb of an L-shaped brass rod the vertical limb of which is held in a stout support. To the upper end is attached a fine waxed cotton thread, which is employed as a means of applying tension and of attaching the recording lever. Loading was somewhat differently effected in different experiments; in some cases the thread attached to the upper end of the muscle passed over a vertically placed frictionless pulley and a weight was fixed to the other end; in other cases the thread was fixed to the lower end of a steel spiral spring suspended vertically over the muscle; finally in those cases where the tension required was very low, the thread was attached to a lever which by means of a weight applied at the axis tended to move upwards, the thread being so fixed that the lever was held horizontal. In those cases in which much tension was employed a length of fine wire connected the muscle to the recording apparatus, in other cases waxed cotton was employed throughout.

The recording apparatus consisted of a small circular plane mirror silvered upon its external surface and rotating around a horizontal axis lying in the plane of its reflecting surface. Care was taken to reduce the friction at the ends of the axis to a minimum. To the back of the mirror was fixed, perpendicular to the surface, a short thin brass wire which served as an attachment for a very light rod made of straw or capillary glass tubing. The length of this was varied according to the magnification required. Its free extremity was attached to the cotton or fine wire fixed to the upper end of the muscle, and so moved with this, producing an angular rotation of the mirror surface proportional to the vertical movement of ascent or descent of its free end, and therefore of the muscle. All that is now necessary is to record the movements of the mirror surface, and this is effected by throwing a beam of light upon the surface, the movements of which are recorded upon a travelling sensitive plate. The general details of the arrangement we adopted are seen from the figure. The light from a bright source is condensed on to a narrow horizontal slit, and that passing through this is collected by a projecting lens, falls on the surface of the mirror and is reflected from this on to a long narrow slit fixed vertically in a light-tight box, inside which the photographic plate, held vertically, travels in a horizontal direction. By means of the projecting lens, the beam of light is carefully focussed so that a sharply defined image of the horizontal slit is



ARRANGEMENT OF MUSCLE VIEWED FROM THE SIDE

RECORDING APPARATUS FROM ABOVE

Fig. 1.

produced on the photographic plate. The magnification of movement attained is calculated by doubling the ratio between the distances of the mirror from the photographic plate and from the connection to the thread respectively.

This method of recording movement has not yet so far as we are aware been applied to physiological purposes, and it seemed to us to offer so many advantages that if it could be readily and conveniently applied it might prove of much value. The work we were commencing was particularly suitable in one respect, because the movements to be recorded were slow. The method has the immense advantage that almost any magnification can be attained, and the higher the magnification required the less becomes the weight of the recording lever. Moreover the lever is moved from its free end, and this in conjunction with the small amount to which the weight of the moving parts may be reduced, eliminates all questions of inertia to the lowest possible point.

The temperature of the muscle was judged by the readings of a delicate thermometer, whose bulb was immersed in the beaker of defibrinated blood and brought to lie as near to the muscle as possible. The record of the temperature at any instant was made by blocking out the light for a time, varying with the rate at which the plate was moving. As a rule each rise of 2° C. was thus recorded in other cases of every 5° C. The actual record thus appears as a break in the continuity of the recording line. In the figures, which are copied from the photographs, these breaks have been omitted, the more considerable changes of temperature only being marked.

Our experiments fall into two groups, the first of which deals with the alteration in length produced as the temperature rises from 0° C. to the onset of heat-contraction, and the second deals with the changes produced from the onset of heat-contraction to higher temperatures. In the first group the changes in length produced as the temperature rises are as a rule quite small in amount, and we have therefore recorded them under a magnification of either twenty or forty-fold, and in some cases of a still higher amount. In the second group the alteration in length is as a rule large and we have consequently only magnified the movement 10 or 15 times[1].

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[1] In the reproduction of our tracings for this paper the original figures have been uniformly reduced to one-half. Any reference to magnification in the text refers to that of the original tracings, and all measurements mentioned also refer to the originals.

III. THE CHANGES IN LENGTH PRODUCED BY A RISE OF TEMPERATURE FROM 0° TO 32° C. UNDER VARYING LOADS.

The experiments we have to record in this connection are of two series. In one set a sartorious preparation was employed, in the other the semimembranosus and gracilis. Owing to the much smaller thickness of the sartorius many disturbing factors are absent in the experiments with it which are present in the other series. To get good results it is necessary to use the upper attachment to the symphysis pubis, and in preparing the lower end to remove, with the tendon, the piece of the tibia to which it is attached.

We may state that as a general result of our experiments we find that with all loads a rise of temperature produces an elongation. The amount of elongation varies directly with the load, the change of

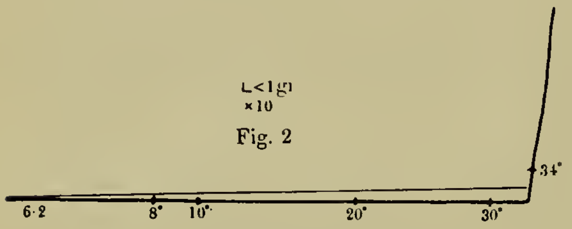


Fig. 2

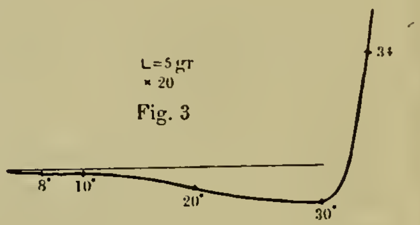


Fig. 3

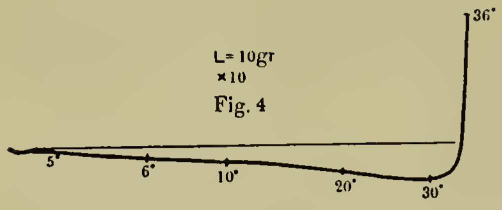


Fig. 4

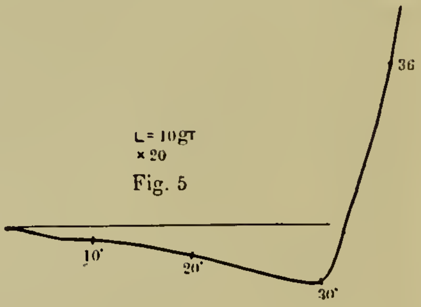


Fig. 5

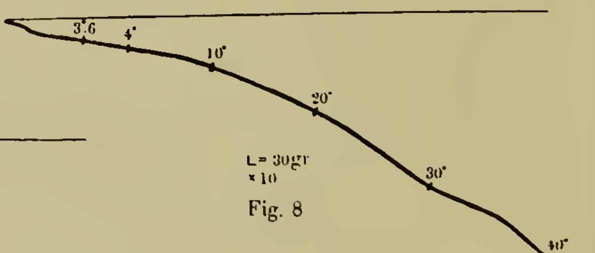


Fig. 8

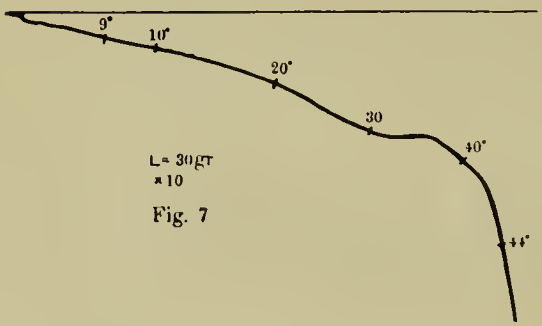


Fig. 7

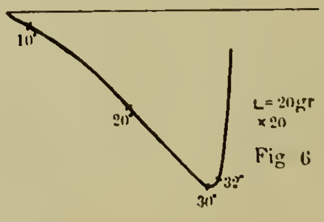


Fig 6

extensibility for a high load being much more marked than for a low load. We have obtained a few exceptions to this general rule, which we will return to later.

In Figs. 2 to 8 we reproduce a series of tracings obtained with gradually increasing loads. In all these the tracings are to be read from left to right. The loads are respectively less than 1 gram, 5, 10, 10, 20, 30, and 30, grams.

In the present connection, confining our attention to that part of the tracing from the start until a temperature of 28° C. is reached we would point out the following:

(i) They all show that elongation occurs as the temperature rises.

(ii) In the earlier tracings, *i.e.* where the load was low, it is seen that the tracing from the start to a temperature of 28° C. is practically a straight line. For the later experiments the curve is convex upwards, though here also the first portion of the curve, up to about 20° C., is a straight line. We may express this in another way, viz.:—

(*a*) For low loads the increase in length is directly proportional to the rise of temperature.

(*b*) For high loads the increase in length is at first proportional to the rise of temperature, but between 20° and 30° C. the ratio of increase of length to rise of temperature gradually increases.

(iii) The rate of increase of length varies with the load, and moreover, the rate of increase of length is very nearly proportional to the load. This is perhaps best seen by a consideration of the following table:

In the seven records the increase of length as the temperature rose from 8° to 28° C. was measured. This is given in column II. Column III gives the original length of the muscle. Column IV gives the increase of length per unit of length, which is obtained by dividing the figures in column II by those in column III. The last column gives the amount of elongation obtained per unit of load, and is obtained by dividing the figures of column IV by their respective loads.

TABLE I.

I	II	III	IV	V	
No. of Exp.	Increase in length for rise of 20°C. in mm.	Initial length of muscle in mm.	Elongation per mm.	Elongation per mm. per grm.	Amount of load
Exp. 98 (Fig. 2)	·0875	24	·0037		Under 1 grm.
„ 89 (Fig. 3)	·1875	32	·00586	·00117	5 „
„ 95 (Fig. 4)	·25	23	·01087	·00108	10 „
„ 88 (Fig. 5)	·25	32	·0078	·00078	10 „
„ 90 (Fig. 6)	·8625	32	·0269	·00135	20 „
„ 92 (Fig. 7)	1·125	28	·0387	·00129	30 „
„ 93 (Fig. 8)	1·175	28	·042	·00140	30 „

In Figs. 9—14 are reproduced a similar series of tracings in which the preparation was a gracilis and semimembranosus. The loads in these experiments were respectively 30, 50, 70, 80, 110, and 120 grams. The magnification employed was 40. On the whole they show the same points as the previous experiments upon the sartorius but with certain striking differences. In the two last figures (13 and 14) the rate of movement of the plate was slower, so that a longer range of temperature is recorded.

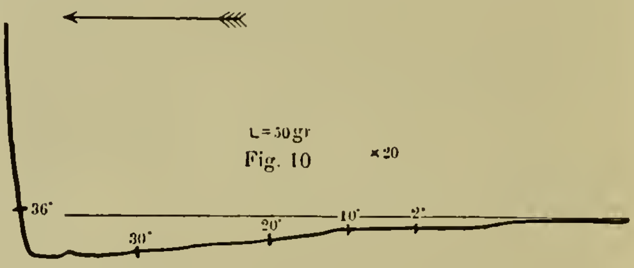


Fig. 10

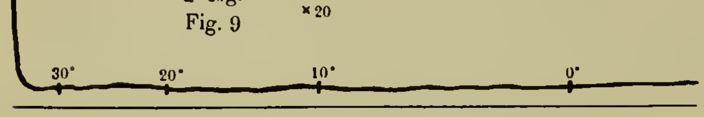


Fig. 9

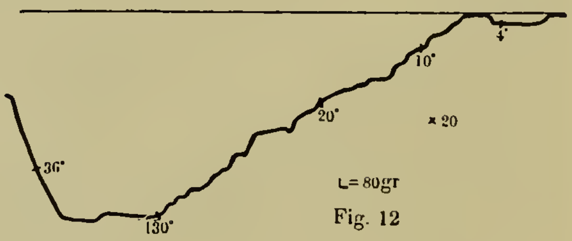


Fig. 12

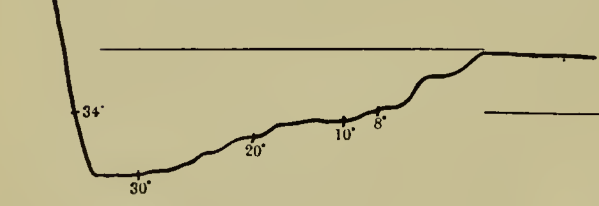


Fig. 11

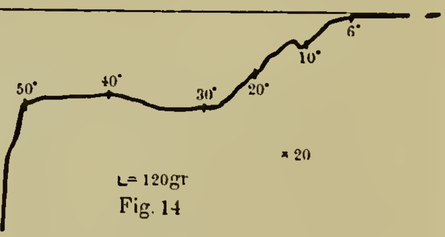


Fig. 14

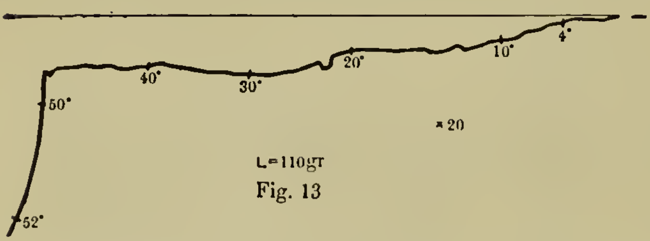


Fig. 13

It is seen from these that a rise of temperature causes an elongation and that, in this elongation there are two very characteristic points to be noted. In Figs. 9, 10, 11 and 12 it is seen that the rate of elongation increases with the load employed, but in the other two it has again diminished although the load has been further increased. Whether the whole of this latter difference is due to the amount of extension previously caused by the load before the temperature was made to rise has still to be decided. The second point to note is, that in most there is a sudden change in the rate of extension at a certain point. The temperature at which this occurs varies to a certain degree in different muscles, but usually occurs from about 8° to 14° C. This sudden change in extensibility corresponds to the somewhat similar phenomenon noted in the sartorius experiments, the only difference being in the question of its suddenness in these experiments. This suddenness of change is in many of our tracings very pronounced, *e.g.* in Fig. 15, where it is seen to occur at 12° C.

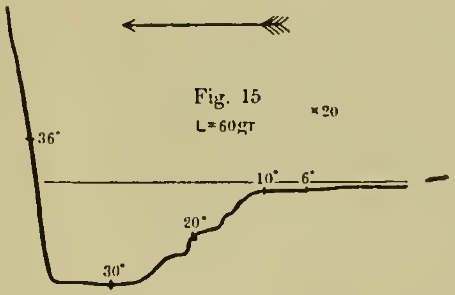


Fig. 15
L=60gr

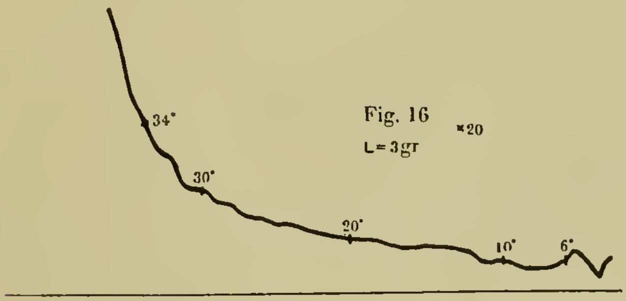


Fig. 16
L=3gr

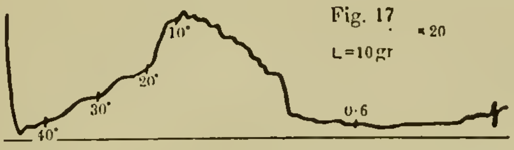


Fig. 17
L=10gr

Some of the differences seen in the two sets of experiments may possibly be due to the fact that in the sartorius experiments fresh diluted ox-blood was employed as the immersion fluid, whereas in the others the preparation was immersed in 0·65 % NaCl solution.

We have already noted that we have observed one exception to the general result that a rise of temperature from 0° to 30° C. causes an elongation. This difference we have found consists in a contraction as the temperature rises if the tension of the muscle be very low. In Fig. 16 we reproduce one of these tracings. The magnification is 40-fold and shows marked contraction as the temperature rises. The preparation is a semimembranosus and gracilis. We have also seen some indication of a similar effect using a sartorius stretched by a very slight load, but although we have made several attempts to obtain a typical tracing from a sartorius, taking all precautions, we have invariably failed. We are inclined to ascribe it to an action of "normal saline" upon the muscle rather than to the simple action of heat. We have determined that it is not in any way a question of alteration in irritability. A sartorius which has lost its irritability by rough handling, or any other means, gives just the same tracing as one possessing normal irritability.

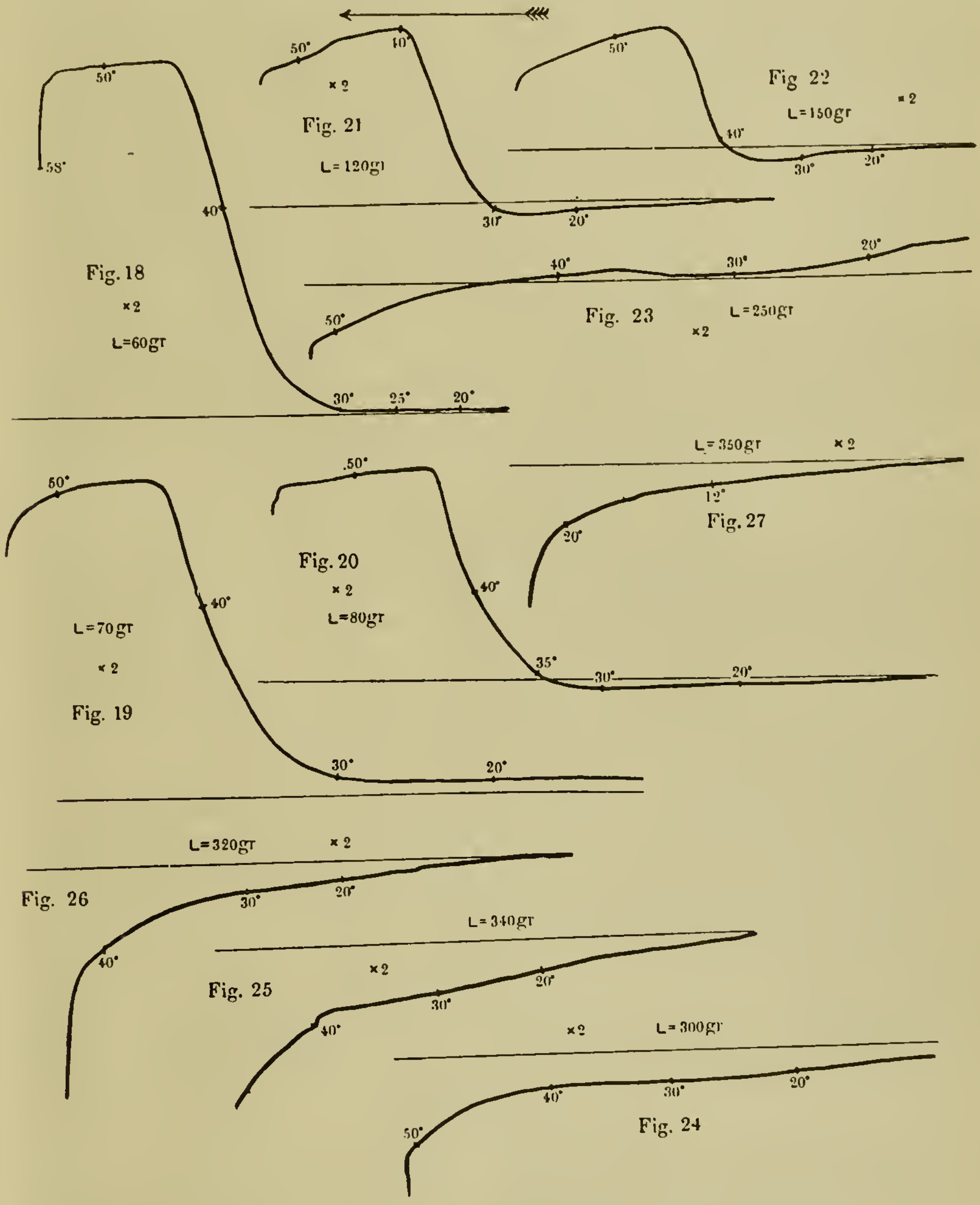
There is one other exceptional result which we might here record. It is given in Fig. 17. The preparation was a semimembranosus and gracilis immersed in 0·65 °/₀ NaCl solution and weighted with a load of 10 g. The record shows a contraction as the temperature rises from 0° C. to 10° C., which is at once followed by an elongation. We have never observed this result a second time though we have attempted to repeat exactly the same conditions.

IV. THE CHANGES IN LENGTH PRODUCED BY A RISE OF TEMPERATURE ABOVE 30° C.

These experiments are directed especially to a study of some of the phenomena associated with the production of a heat-contraction, and therefore particularly include the questions, of the temperature at which it commences, the way in which it is modified by varying the load, a measurement of the absolute force which it can exert and its true causation. The experiments have been carried out on the same two preparations as those in the previously described experiments. Figs. 18—27 are tracings of the results obtained from gracilis-semimembranosus preparations, and throughout the series the load is a gradually increasing one. The magnification is 6·7. In some of the tracings the temperature record is every 5° C., in others every 10° C.

In an examination of the series the first point that we may note is the temperature at which the first sign of shortening is to be detected. This is seen to be somewhat variable for individual muscles. In Fig. 22

it is seen to be delayed as late as 37° C. In Fig. 19 it is seen to begin as early as 28° C. We have performed many experiments in order to determine exactly the point at which shortening commences



and have found that it is a very variable one for different muscles. The conditions which we have been able to show produce differences in the result are; (1) differences in individual muscles, (2) the rate at which the temperature rises, and (3) the amount of the load.

Muscles from different frogs give variable temperatures of heat-contraction which may extend over 2° C. If the rise of temperature be very slow we have generally, though not universally, found that the earliest sign of contraction is noted at a lower temperature. Lastly many of our experiments tend to show that with very high loads the first sign of a contraction is only to be noted at a much higher temperature. This latter point, we think, is readily explicable by the fact, that as well as the question of actual shortening of the muscle there is the second factor to be taken into account, viz., that at the temperature of heat-contraction there is a very sudden change in extensibility which becomes markedly increased. As a general statement we may say that the usual temperature at which contraction first commences is about 32° C., and that it is usually very well marked at 34° C.

A study of the actual contraction itself offers points of interest in connection with the amount of contraction, its force, and the rate at which it is effected. In Fig. 18 the load was 60 g., and the muscles contracted $\frac{4}{9}$ ths of their initial length. In Fig. 21 the load was 120 g., and the muscles contracted $\frac{2}{9}$ ths of their initial length. We have in many cases with a low load found that a muscle may contract to less than half its original length. With a low load, the amount of the contraction may be distinctly greater than the amount produced by maximal tetanisation. The rate of the contraction is also found to vary, the higher the load the slower becomes the contraction and the quicker it ceases. As the load is increased the amount of the contraction falls until we see in Fig. 23 that it is almost completely prevented, and in Fig. 24 that it is quite absent.

Associated with the diminution in the amount of contraction as the load is increased it becomes of interest to determine the total amount of work performed by the muscle in each of the cases. The results are not strictly comparable because of the necessity of employing a fresh frog for each experiment, so that in order to get rather more comparable results we have in each case weighed the muscle and have thus been able to express the result as so many grm. mm. of work per 1 grm. of muscle. The results are presented in the following table.

TABLE II.

	Total work in g.mm.	Weight in grms.	Work per 1 g. of muscle-substance
Exp. u (Fig. 18)	725·37	·386	1879·2
,, v (Fig. 19)	720·89	·342	2107·9
,, w (Fig. 20)	573·13	·332	1726·3
,, z (Fig. 21)	752·24	·385	1953·9
,, α (Fig. 22)	671·64	·370	1815·3
,, β (Fig. 23)	55·97	·480	116·11

These experiments also enable us to determine the absolute force of the contraction. Thus in Fig. 23 the weight of the muscle was 0·48 g., its length was 29·25 mm. and therefore its average diameter was 15·407 sq. mm. (taking the sp. gr. at 1·066). The load which almost prevented the contraction was 250 g. and consequently the absolute force per mm. of muscle, as calculated from this particular preparation, is somewhat above $\frac{250}{15{\cdot}407} = 16{\cdot}23$ grms. per sq. mm. A similar calculation based on the result in Fig. 24 gives it as 20·04 grms. per sq. mm. Other calculations, but from the sartorius, based on the experiments which yielded Figs. 7 and 8 gave the result of 19·47 grms. per sq. mm.

If the load be still further increased, as in Figures 25, 26, and 27, a new change becomes apparent, for it is found that at the temperature at which heat-contraction usually appears, or at a temperature a little higher, there is a sudden change in the rate of elongation, which is in many cases very marked (Fig. 25). This change of rate is always in the direction of showing an increase in the extensibility. If the load be pushed to a still higher degree we found that the influence of a temperature rise was to lead to so marked a degree of extensibility as to quickly lead to a rupture through the muscle substance at quite a low temperature (24° C., see Fig. 27).

With regard to the point at which heat-contraction ceases we find that there is no connection between it and the temperature at that instant. We have previously shown that a muscle may be made to complete the whole of its heat-contraction at the temperature at which that contraction first shows itself. We also showed the time required to complete that contraction was much less at a temperature of about 40° C. than at one of 34° C. Consequently even with a rapidly rising temperature we never find that the contraction increased much after 40° C. had been reached.

In Figs. 28—32 we have a similar series, where the preparation employed was the sartorius, which show practically the same points as in the previous set. In Fig. 31 where the load was 25 g. the contraction is nearly abolished, and in Fig. 32 where the load was 30 g., it is completely prevented, and replaced by an increase in the extensibility. The results by the sartorius preparations are certainly better in that being so thin the temperature at each instant is much more nearly the same throughout the whole muscle. This is, perhaps, particularly of value in the experiments with high loads.

We may further point out that some of these later tracings are supplemented by earlier ones, *e.g.* Figs. 7 and 8, where however the magnification is different (20 instead of 6·5).

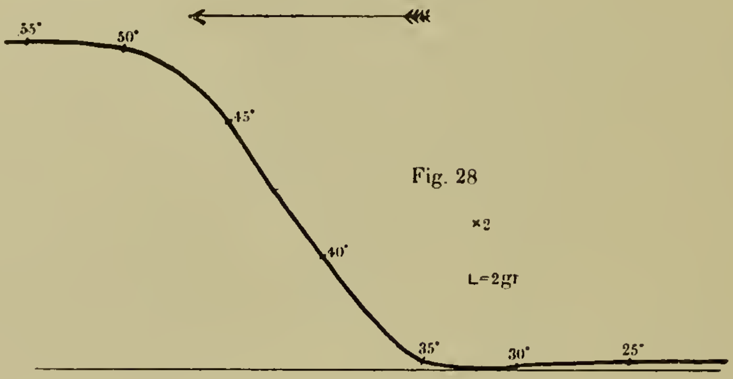


Fig. 28

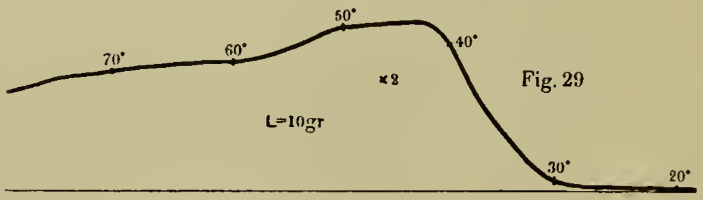


Fig. 29

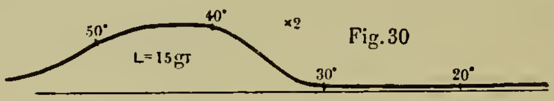


Fig. 30

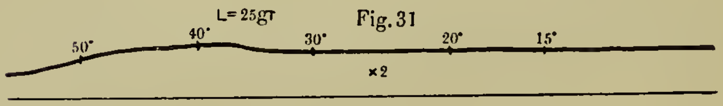


Fig. 31

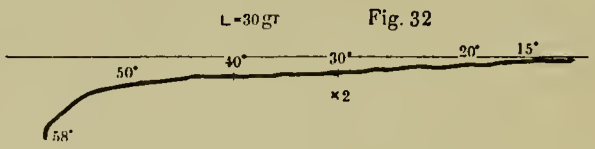


Fig. 32

To compare with the previous set of experiments upon the semi-membranous and gracilis the following table gives the amounts of work performed in the experiments of Figs. 28 to 31.

TABLE III.

	Amount of load in grms.	Amount of work in grm. mm.
Exp. *d* (Fig. 28)	2	20·3
,, *h* (Fig. 29)	10	53·7
,, *i* (Fig. 30)	15	17·9
,, *m* (Fig. 31)	25	3·73

From the two last tables and from a general consideration of all our experiments it is seen that at first as the load is increased the amount of work performed by a muscle as it produces a heat-contraction also increases until an optimum load is reached, at which a maximum amount of work is performed. If the load is progressively increased beyond this point, the amount of work gradually diminishes until at last a load is reached at which no work is performed.

There are still one or two other points to be noted in the tracings, and especially with regard to the results observed when the temperature has passed above 40° C., *i.e.* when the muscle has passed into a state of complete heat-contraction. We have previously recorded the fact that where the load is very low, *e.g.* under one gram, that there are produced three successive further contractions occurring respectively at about 48°C., 56°C., and 65°C. (see Fig. 33). It now remains to consider what are the results when the load is increased. A very slight increase of load suffices to prevent the occurrence of these later contractions. They possess, that is, so small an amount of force that they are unable to make themselves felt in a muscle which is already contracted to a considerable degree. In most of the tracings it is seen that where the load is low, but above one or two grams, the tracing beyond about 40° C. is a straight line parallel to the zero abscissa (Fig. 28). When the load is further increased (Fig. 29) it is seen that, as soon as the main contraction is fully completed, an elongation tends to set in, which becomes much more pronounced at those temperatures at which further contractions were produced by muscles in which the tension was reduced to a minimum (see also Fig. 30). With still higher loads we find yet another result obtaining, viz., a rupture of the muscle. This is well seen in Figs. 19 to 27. As the load increases the extension produced by a rise of temperature above that at which contraction ceased becomes more and more pronounced, and leads ultimately to a rupture of the muscle. This rupture is more particularly liable to occur at those temperatures—or rather slightly above them—at which heat-contractions are produced. When they occur they are found to divide the muscle through its substance, and not at the attachments to the tendon, if sufficient attention has been paid in the preparation of the muscle to the protection of the tendinous attachment and the adjustment of the thread without injury to the tendon.

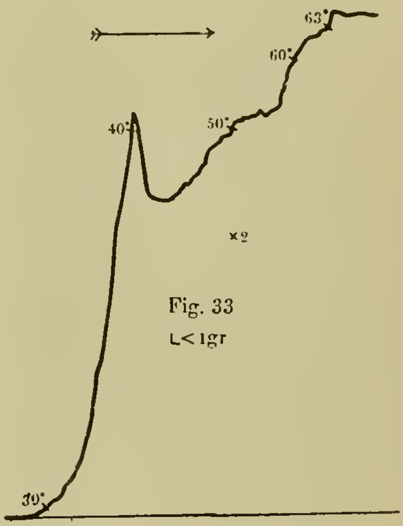


Fig. 33
L<1gr

V. SOME EXPERIMENTS UPON THE CHANGES IN ELASTICITY AND EXTENSIBILITY OF INDIARUBBER CAUSED BY A RISE OF TEMPERATURE.

In the course of our experiments upon muscle we felt the great desirability of being able to contrast them with control experiments of a similar nature carried out upon some elastic and extensible substance other than an animal tissue. The first substance that suggested itself was gutta-percha, and upon the effect of heat upon this we were only able to find some experiments by Joule[1] and by Schmulewitsch[2]. In 1857 Joule published some results obtained with vulcanized indiarubber. He found that when it was stretched by a weight its temperature rose, and when the load was removed its temperature fell. This led him to an examination of the effect of heating a loaded piece of indiarubber. He found that if a piece was stretched to double its length that it contracted about $\frac{1}{10}$th by raising the temperature to 50° C. This shortening effect was found to increase in amount with the stretching weight employed, exactly in accordance with the heating effects observed with different stretching weights. It thus behaved exactly in conformity with Thomson's theoretical considerations upon the shortening or lengthening of a body when heated, if that body develops heat or loses heat when it is stretched.

Further experiments in this direction were carried out by Schmulewitsch, who confirmed Joule's observation of the contraction of indiarubber as the temperature was raised, but further showed that if it was unstretched the effect of heat was to produce an elongation. He pointed out that there was a certain tension for a given piece of rubber at which it neither elongated nor contracted when warmed. He showed too that if subsequently cooled the rubber only returned to its initial length after a prolonged interval of time, and that if the temperature had been taken much above 50° C. a permanent change was always set up which expressed itself as a marked increase in extensibility.

In our experiments we have throughout employed pure non-vulcanized indiarubber. We have confirmed the results of Joule and of Schmulewitsch and extended them in certain directions. Figs. 34 to 38 give some of the typical records we have obtained. The magnification in Fig. 34 was 107·36 times, in the others 15. In Fig. 34 the tension employed was only just sufficient to keep the piece of rubber straightened out, and we see that as the temperature rose it elongated, though the amount of that elongation was very small. We had to take a longer piece of rubber than in any of the other experiments, and the magnification had to be very high in order to get any well-marked elongation.

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[1] *Proc. Roy. Soc.* VIII. p. 355.

[2] *Vierteljahrsschr. d. naturforsch. Gesell. in Zurich.* XI. p. 201, 1866; *Bull. de l'Acad. de St Pétersbourg* XIV. p. 517, 1870; *Centralb. f. d. mediz. Wiss.* V. p. 133, 1867.

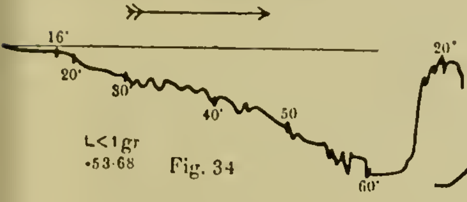
Fig. 34

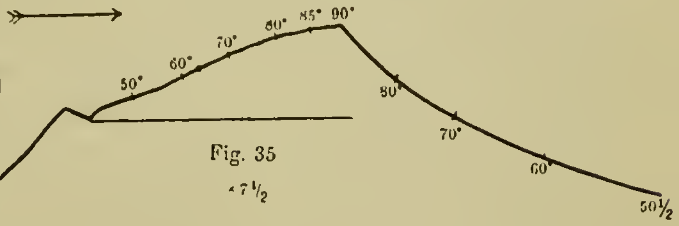
Fig. 35
× 7½

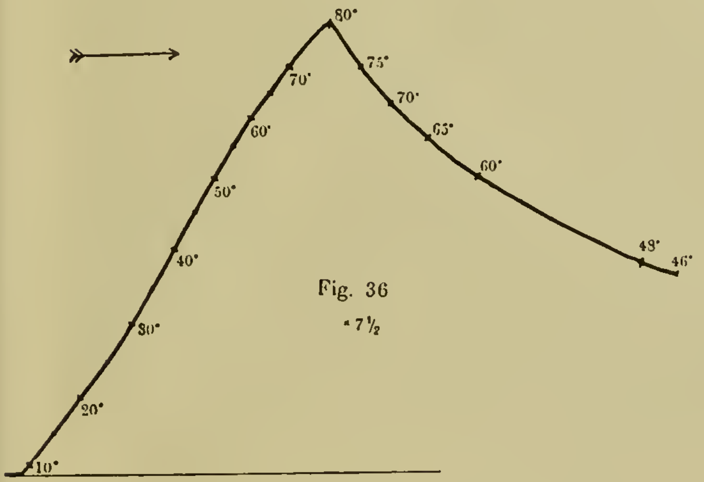
Fig. 36
× 7½

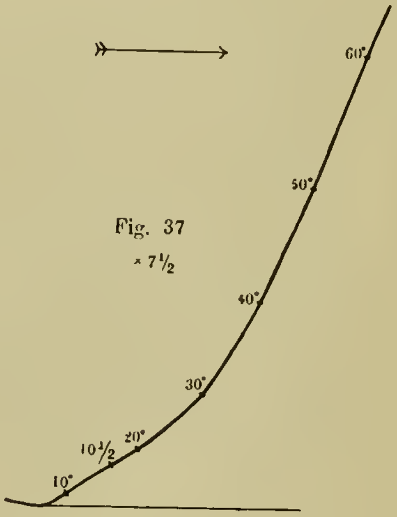
Fig. 37
× 7½

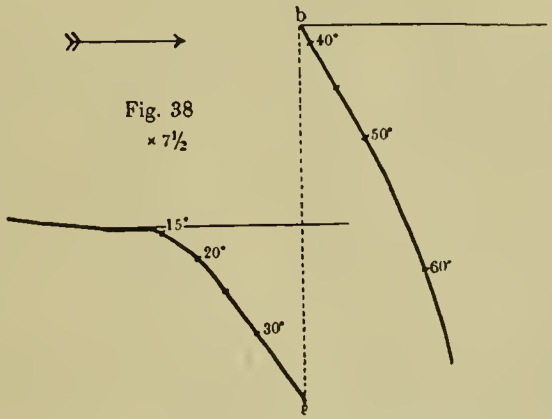
Fig. 38
× 7½

In the next three experiments, 35, 36 and 37, the rubber was previously stretched. In 35 it was stretched from 2 cm. to 4 cm., which required for the piece of rubber we used a load of 94 grams. In 36 a piece of the same rubber was stretched from 2 cm. to 7·1 cm. requiring a load of 164 grams. In 37 another piece was stretched from 2 cm. to 10 cm. requiring a load of 240 grams. From an examination of these tracings it is at once seen that an increase in the tension produces a greater contraction with a given rise of temperature. This is well brought out in the accompanying table. Passing next to a still higher tension we found that the contraction began to diminish in amount until at last it resulted in an elongation. This last result is well seen in Fig. 38. There 1 cm. of rubber was stretched to 8·2 cm. requiring a load of 384 grams, and we found that this produced a very marked elongation, ultimately resulting as the temperature rose above 60° C. in rupture.

These results we have collected together in the form of Table IV.

In the first column is the length of rubber taken; in the second, the length of the rubber under the influence of the load; in the third, the load applied; in the fourth, the amount of alteration in length produced by a rise of temperature through 30° C. expressed in cms. per cm. of unloaded rubber, elongations being shown by a $+^{\text{ve}}$, contractions by a $-^{\text{ve}}$ sign; in the fifth column is the change in length in cms. per 1 cm. of length of the loaded rubber at the start of the experiment.

TABLE IV.

	I	II	III	IV	V	VI
	Length in cms. or rubber $=l$	Length in cms. when stretched $=l'$	Load in grams $=L$	Change in length per cm. of original length $=\frac{dl_1}{l}$	Change in length per cm. of stretched rubber $=\frac{dl_1}{l_1}$	Amount c work in grm. mm
Exp. 33 (Fig. 34)	5·8	5·8	·0	+ ·00305	+ ·00305	0·0
„ 41 (Fig. 35)	2·0	4·0	94	− ·0416$\dot{6}$	− ·0208$\dot{3}$	+ 7·8
„ 42 (Fig. 36)	2·0	7·1	164	− ·1466$\dot{6}$	− ·04133	+ 38·1
„ 43 (Fig. 37)	2·0	10·0	240	− ·2416$\dot{6}$	− ·048$\dot{3}$	+ 116·0
„ 44 (Fig. 38)	1·0	8·2	384	+ ·466$\dot{6}$	+ ·057	− 179·2

An examination of the Vth column of this table tends to show that the amount of contraction produced increases with the load very rapidly, and this becomes all the more striking if we view it in conjunction with the fact that with the higher tensions the transverse sectional area of the pieces of rubber become proportionately diminished. The transverse sectional area does not diminish proportionately to the amount of elongation produced but at a somewhat less rate, for as is well known the volume increases as the rubber is stretched.

We may very readily apply the main correction to the figures in column V. required by the change in sectional area, if we act on the supposition that the amount of power to contract, or elongate, is directly proportional to the transverse sectional area. That is, we leave out of consideration the increase in volume due to an elongation, and the changes in sectional area occurring during the small alterations in length, during the actual experiment. These two factors would only produce quite a small effect on the resulting figures, and moreover they would act in opposition to one another in the three middle experiments, but in the same direction in the first and last. If this be done in this way, we shall of course get the figures of column IV. which may therefore be also taken to represent the amounts of contraction or elongation in cms. per 1 cm. of length per unit of transverse sectional area, taking this last unit as that of the sectional area of the piece of rubber when unloaded.

In the last column (VI.) are placed the amounts of work either performed by the rubber (positive work) or performed by the load upon the rubber (negative work). These are of course obtained by multiplying the load by the change in length.

VI. CRITICISM AND CONCLUSIONS.

We have carried out several experiments with the special object of determining whether the statement made by Schmulewitsch that irritable muscles gave a contraction followed by a relaxation when the temperature rose to 28° C. and again when it attained 32° C. We have invariably found that such does not occur, and thus can confirm Gotschlich upon this point. Irritable and non-irritable, curarised and non-curarised gave always the same result.

The fluid in which they were immersed also made no difference, for the same result was obtained when "normal saline" was employed as when diluted blood was used. We have made some experiments to directly test whether the contraction at 30° C. described by Gotschlich as sometimes observable, could be always obtained if the load was sufficiently decreased. We could always observe a very small contraction at 30°, either in salt solution or in blood, but relaxation never followed on cooling when the load was under 1 gram. If after such a contraction the muscle be extended, passively, to its initial length a second warming to 30° C. caused a fresh contraction. The lower limit at which this contraction was observed was 29° C.

Our conclusions may be briefly summed up in the following statements:—

1. As a muscle is warmed from 0° C. to 30° C. it elongates, becoming more extensible for all loads.

2. The amount of the elongation varies directly with the load.

3. The rate of elongation is almost constant for low loads. For higher loads there is either a gradual increase in the rate as the temperature rises, or there is a sudden alteration in the rate at a definite temperature, usually at about 12° C.

4. From these we may deduce the fact that a muscle becomes more extensible as the temperature is raised from 0° C. to 30° C.

5. At about 34° C. heat-contraction sets in. The temperature is not exactly the same for all preparations.

6. The amount of work done during this contraction increases at first rapidly, with the load, then diminishes as the load is further increased until at last it is reversed, negative work, *i.e.* work performed by the load upon the muscle, suddenly setting in as that temperature is reached.

7. The absolute force of that contraction amounts to about 20 grms. per sq. mm. of transverse section.

8. At the temperature of heat-contraction there is produced a sudden change in extensibility, in the direction of increase.

9. At each of the other temperatures at which proteid coagulation occurs in a muscle, *i.e.* at 47° C. and 56° C., there is a further sudden increase in its extensibility.

10. All these changes are physico-chemical in nature, they are quite independent of irritability, are produced just as well in a thoroughly fatigued muscle, even by one in a fairly advanced stage of rigor-mortis. Curare makes no difference to the result.

11. Unloaded pure indiarubber elongates as its temperature is raised.

12. If the load be increased it contracts, and the amount of contraction at first increases with the load, and then decreases.

13. If the load be still further increased elongation is produced by a rise of temperature. There are, consequently, two loads at either of which the length remains constant as the temperature rises, the one is low, the other very high.

[The expenses of this research have been defrayed partly by a grant from the British Medical Association, partly by one from the Government grant of the Royal Society.]

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THE ENUMERATION OF BLOOD-PLATELETS.

By T. G. BRODIE, M.D., *Lecturer on Physiology,* and A. E. RUSSELL, M.B., *Medical Registrar, St Thomas's Hospital Medical School.*

(*From the Physiological Laboratory, St Thomas's Hospital Medical School.*)

It is well known from the observations of Bizzozero, of Eberth and Schimmelbusch and of many others that the platelets of the blood immediately or almost immediately that the blood is shed, adhere to one another and to foreign bodies. In the estimations which have been made upon the number of platelets, this fact does not appear to us to have been taken sufficiently into account. Thus if the Thoma-Zeiss pipette be used in the usual way, during the time the blood is in contact with the glass, a great many of the platelets become adherent, so that the number found will always be too low. We have therefore made trial of numerous fluids with the aim of finding one or more in which the platelets do not stick to one another or to the red or white cells or to the glass, at any rate for a sufficient time to allow one or two enumerations to be made.

In mixing the blood with the diluting fluid we have a choice of the two methods of first placing the fluid on the finger, which has been previously thoroughly cleaned, and then pricking through the drop of fluid, or of adding a drop of blood collected on the finger to a sufficient quantity of diluting fluid held in some convenient glass vessel. The first method is undoubtedly best where the end aimed at is the fixing in as normal a state as possible, the second plan is more convenient in many respects for it allows of better mixing by stirring and one has more control over the relative amounts of the two fluids. It is the plan we have throughout adopted as a general one, but has been controlled by the first method.

We may now proceed to an enumeration of most of the various fluids we have tested which we will group into series for the sake of convenience, the grouping being either according to the nature of the solution or according to the results upon the platelets.

I. Solutions of many neutral salts have been tested, such as:—sodium chloride, magnesium sulphate, ammonium sulphate, sodium sulphate, potassium sulphate, potassium iodide, sodium iodide, potassium and sodium acetate. These have been tested in solutions of different strengths starting always from one approximately isotonic to the red-cells and then increasing the strength. They none of them were found to fulfil the necessary conditions. In many of them it was found that the change of the platelets was considerably delayed, this varying with the solution, but in all cases, some platelets were found fixed to the cover-glass immediately the preparation was examined.

II. Various fixing reagents recommended for the examination of the blood were tried such as Hayem's fluid, Ferrier's solution (alcohol 50 c.c., glycerine 200 c.c., water 150 c.c., fuchsine 1 g.), Toison's fluid, formic aldehyde of different strengths, Pacini's fluid, Afanassiew's fluid (·6 °/₀ NaCl and ·6 °/₀ dry peptone in water, to which is added 1 or 2 parts per thousand of methyl violet), &c. All of these were again found to be imperfect though in very different degrees. The best of these was Afanassiew's fluid, though here a number of the platelets were found adhering to the cover-slip or slide.

III. Solutions of substances which prevent coagulation were also tried, *e.g.* ammonium, potassium and sodium oxalate solutions of various strengths, sodium fluoride solutions, potassium and sodium citrate solutions, or commercial peptone solutions. All of these showed a distinct advance over previous solutions employed, and this was especially the case with the oxalate and fluoride solutions. The citrates were the least effective. A good deal appears to depend upon the ratio of the volume of diluting fluid to the volume of blood taken. It is always better to dilute the blood considerably.

IV. A miscellaneous group of fluids such as, Müller's fluid, borax solutions, perchloride of mercury solutions, formic aldehyde or acetone of different strengths. Some of these fixed the platelets well, but all were found of no use in that platelets did not remain freely movable in them.

V. A solution of caustic potash of 33 °/₀ was tried. In this fluid the platelets are better preserved than in any of the fluids in which we have examined them. Their shape is very clearly seen though, as a slightly granular appearance is given to them, it is probable that they are not entirely unaffected in histological appearance. The fluid is however of no use whatever for our purpose because of its destructive effect upon the red cells. The drop of blood is at once converted into an extremely viscid jelly-like mass which completely resists any attempt at breaking it up for uniform distribution.

VI. Thinking that perhaps we might prevent any tendency to adhesion if we brought the blood into a viscid solution we turned our attention to such fluids as egg-white, serum, syrup, gum-solutions, Farrant's solution and glycerine. We found that our conjecture was in a great measure carried out. One of these, glycerine, is by far the best fluid we have found. It answers perfectly to our test conditions so far as the platelets are concerned. They show no tendency to adhere to the glass nor to run into clumps but remain freely movable in the solution. The stronger the solution of glycerine the better is the effect with regard to the platelets, and we have usually employed a mixture of equal parts of glycerine and 2 °/₀ NaCl. It unfortunately has the great disadvantage that it acts upon the red cells, dissolving out the hæmoglobin, so that after a time the stroma becomes almost if not entirely invisible. The action however takes time and it is quite possible to get an enumeration completed before the fluid has so attacked the red cells that there is a danger of missing them.

In our further work we limited ourselves to those fluids in which, as we had found, the platelets are freely movable, *i.e.* to solutions of glycerine, of oxalates, or of fluorides.

In the next place, it was requisite to stain the platelets deeply so that they could be easily counted. When we were dealing with unfixed platelets, this we found was not an easy matter. We tested the various stains that have been recommended, such as methyl-violet, gentian-violet, rosaniline-sulphate[1], but though they are stained by them the amount of staining is not great. We have tested a number of stains to see if fresh platelets are stained by them, such as eosin, fuchsin, aurantia, methyl-green, magenta, aniline blue-black, congo-red, indulin, iodine-green, toluidene-blue, dahlia, methylene blue, but of these only two stain platelets at all deeply, viz. iodine-green and dahlia, and of these two dahlia staining is much the deepest and therefore preferable for our purpose.

We have, when possible, employed a very strong solution of dahlia in glycerine, or diluted glycerine. As far as the platelets are concerned a solution which gives excellent results is one made up of equal parts of glycerine saturated with dahlia, and 2 °/₀ NaCl.

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[1] All of these together with the other stains mentioned were obtained from Grübler.

In such a solution we have endeavoured to stain the stromata of the red corpuscles by the addition of some other stain, but invariably found that the dahlia was completely thrown out by the new stain or so largely precipitated that the fluid now only tinted the platelets to a very slight degree.

We attempted also to find some substance, which, added to the solution would fix the stromata, and so allow them to be stained. Of the many substances that we tried, alcohol, borax, oxalates and fluorides alone proved of any service. But alcohol, if sufficient is present to allow the stromata to stain with dahlia, alters the platelets so that they will not move freely through the fluid. When sufficient borax solution is added, the red cells begin to take up some of the dahlia (or iodine-green) and then appear as a ring of deeply stained matter close under the outer margin of the red-cell but separated from the margin by a narrow clear uniform layer; but in this case either the platelets do not stain, or are so affected that they are not freely movable. The solution also takes up much less stain than one free from borax.

The oxalates have yielded rather better results than the fluorides. We have already seen that simple solutions of oxalates and fluorides preserve the platelets and prevent them from becoming sticky, but that they are not so good in this respect as glycerine solutions. We thought that by employing oxalate solutions, we might be able to so diminish the action of the glycerine on the red corpuscles that the hæmoglobin would not so readily be dissolved out, or on the other hand might so affect the stroma that it would stain with dahlia. We find that the latter is the case more particularly if a little absolute-alcohol be added to the solution as well.

We find in one solution that a very characteristic appearance is produced. The solution has the following composition:—

Glycerine	25	c.c.
Absolute alcohol	12·5	c.c.
Distilled water	62·5	c.c.
Ammonium oxalate	1·0	grm.
Sodium chloride	1·5	grm.

Notwithstanding its high percentage of salts the red corpuscles swell up and become spherical, and if the corpuscles be watched during this process, which occurs with very different rates for different corpuscles, it is seen that at first there appears to be a diffuse staining which makes the corpuscle stand out of a much deeper colour than the others. Its diameter is also less. A little later if focussed equatorially the appearance is of a deeply stained rim of uniform thickness lying just inside the edge of the corpuscle with a narrow clear zone separating it from the periphery. This rim however is not continuous but is broken at quite regular intervals by clear spaces. If now the adjustment be altered until the surface of the corpuscle is in front it is seen that this broken rim is caused by a regular network of deeply stained substance, the meshes of which are fairly large. The central part appears quite homogeneous and unstained. The network exists as a hollow spherical shell lying beneath the membrane and of some little thickness. Thus the cell-membrane of the corpuscle stands out very clearly and distinctly as a separate unstained structure. That in this shell of stained matter we are dealing with an artefact becomes clear from the variations observed in successive preparations. Differences in the size of the meshwork are observed in different specimens, although in any one specimen the size is wonderfully regular. We are inclined to think that the part actually stained is the stroma and that the peculiar arrangement of this in the form described is due partly to the tearing of the stroma which must occur when the corpuscle is distended to a sphere.

Before we proceed to a statement of the number of platelets present in the blood according to our own enumerations it will be best to consider figures given by some other observers. Thus Hayem[1] gives the number as 255,000 per cub. mm.; Afanassiew[2] gives from 180,000 to 300,000 per cub. mm.; Fusari[3] found them to be from 180,000 to 250,000 per cub. mm.; Prus[4] finds 500,000 per cub. mm. There is thus found to be a considerable amount of variation in the results obtained, and much of this is due, undoubtedly, to the different methods employed. Thus Hayem, Afanassiew and Fusari, all used the Thoma-Zeiss pipette and therefore must have obtained figures which are too low. Prus's figures are much more reliable, for his method was to thoroughly clean the finger and then place on it a drop of a modified Flemming's solution and prick through this. The solution employed has certainly considerable disadvantages, and we find that again Prus's figures are too low, though much nearer the truth than the others. Muir[5] fully recognises that the measurement of the blood by a pipette possesses the fallacy that some of the platelets will adhere to the glass tube but thinks these may be quite neglected if all the operations are carried out rapidly. We cannot agree with him that the use of the Thoma-Zeiss pipette is permissible in this connection, no matter how rapidly the mixing be effected. Muir[1] gives as an average number for a normal individual, 200,000 to 250,000 per cubic mm.

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[1] Hayem. *Du Sang.*

[2] Afanassiew. *Arch. f. klin. Med.* xxxv. p. 217. 1884.

[3] Fusari. *Arch. per. le Scienze med.* x. p. 235.

[4] Prus. *Medycyna* 1886, No. 39 and 40 (abstracted in *Centralb. f. klin. Med.* 1887, p. 469).

[5] *Journal of Anat. and Physiol.* xxv. p. 259. 1891.

The enumerations which we may now give are all carried out upon the lines worked out as above. In most cases the mixing fluid has been placed upon a glass slide and then brought down upon the drop of blood as soon as it appears from the prick. It is important that the blood should flow from the prick rapidly and without the necessity of applying pressure. In all cases before the enumeration we have ascertained that the platelets move freely across the field of the microscope. This is all the more easy in the viscid glycerine solutions where the formed constituents come to rest slowly. In successful cases the platelets are found to be uniformly distributed through the preparation and never in clumps or groups. If such has occurred the preparation must be rejected as unreliable. The counting is carried out under a $\frac{1}{12}$th, the number of red corpuscles and of platelets in a series of fields, taken quite at random, being counted, until about three or four hundred red corpuscles have been seen. The ratio of platelets to reds is then calculated and from this result the number of platelets per cubic mm. The solutions used in the following cases have been various but all contained glycerine in various strengths. The staining was with dahlia.

The ratios of platelets to red corpuscles come out as

1 : 8·0, 1 : 9·2, 1 : 9·0, 1 : 7·0, 1 : 9·4

which gives an average of 1 : 8·5.

The red corpuscles numbered on an average 5,400,000 per cubic mm., which gives for the platelets 635,300 per cubic mm.

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[1] *loc. cit.* p. 273.

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THE DETERMINATION OF THE COAGULATION-TIME OF BLOOD.

By T. G. BRODIE, M.D., *Lecturer on Physiology*, AND A. E. RUSSELL, M.B., *Medical Registrar, St Thomas's Hospital Medical School.* (One Figure in Text.)

(*From the Physiological Laboratory, St Thomas's Hospital Medical School.*)

THE question of the determination of the time the blood takes to clot is one which has always shown difficulties whenever attempts have been made to obtain time measurements of any accuracy. We may first state briefly the methods we have tried and which have not given us satisfactory results, in that successive observations taken at intervals of 5 to 10 minutes have not given concordant results.

(1) Wright's[1] method, in which several glass tubes of narrow bore are filled with the same amounts of blood, and the time determined when the blood cannot be blown from the tube, or if blown out on to filter paper shows a clot.

(2) Vierordt's[2] method, in which the blood is drawn into a capillary tube in which a white horse-hair is placed, and the coagulation-time taken as that at which the blood adheres to the hair when it is moved.

(3) Several pieces of very fine capillary glass tubing of the same bore and about two inches in length were half filled from a single drop of blood and kept at constant temperature. From time to time one was taken and its contents blown out into a beaker of water or of saline solution. If still fluid the blood collects into a drop or forms a ring, but if clotted it remains as a perfect cast of the tube from which it has been blown. Capillary tubes of very various sizes were tested. The method was unsatisfactory because on many occasions some of the tubes still contained fluid blood when one or more had already clotted.

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[1] Wright. *Lancet*, 1893, Dec. 2. p. 1390.

[2] Vierordt. *Arch. f. Heilk.* xix. p. 193. 1878.

(4) A piece of thick-walled glass tubing of 0·5 mm. bore was taken and blood drawn into it in the following way. A column of blood extending up the tube for 1 cm. was taken, and then removing the tube from the drop of blood this was moved up 2 or 3 cms., then a second column of blood of 1 cm. and so on. In this way was obtained a long piece of tubing in which were about 12 little columns of blood, each of 1 cm. in length separated from one another by intermediate air-spaces. The tube was then cut into pieces midway between each mass of blood and from time to time one of these was blown into water and observed as in the previous method. We had expected to find that, if the rate of clotting is in any way dependent upon the number of platelets present, that the lowest columns of blood would clot before the higher ones, for owing to the great stickiness of the platelets the lower part of the tube soon becomes coated with a layer of them, derived from the blood forming the upper columns. This was not however found to be the case.

(5) A piece of capillary tubing 0·5 mm. bore was taken, and a column of 5 cms. of blood drawn up to a marked position in the tube. Every 15 secs. this was moved 1 mm. down the tube until the time was found at which it could no longer be moved.

(6) A drop of blood was taken and kept at constant temperature in a small moist chamber and from time to time a piece of very fine capillary tubing was brought in contact with the edge of the drop and observation taken as to whether blood or serum ran up the tube. This plan yielded much the best results we had so far attained, but yet was not completely satisfactory.

The method which has given us satisfactory results is as follows. A deep air-chamber *AA* (cp. Fig. 1) closed below by a glass plate *E* upon which lies a layer of water *M* is surrounded by a water-jacket

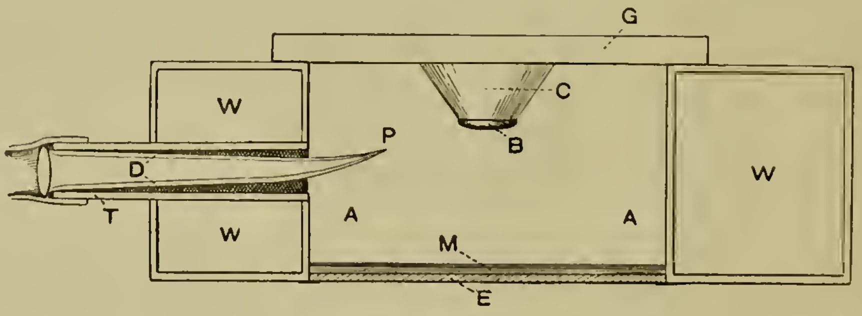

Fig. 1.

WW, its upper orifice being closed by a movable piece of glass slide *G* to the centre of which is cemented an inverted truncated glass cone *C*. Inflow and outflow tubes to the water-jacket enable the temperature of the air-chamber to be varied at will. These tubes are not represented in the diagram. A metal tube *T* pierces the water-jacket, and to its interior is fitted a glass tube *D* drawn out gradually to a fine orifice at *P*. This orifice lies below the lower surface of the cone *C* but is directed towards it[1].

In using the apparatus the glass plate and cone are removed and the lower surface is dipped carefully into the drop of blood so that the whole of the surface is exactly wetted by the blood. In this way a drop of blood is picked up, always of approximately the same size. It is then placed in position in the air chamber. By gently blowing through the tube *D* the blood is set in motion and can be readily observed under a low power of the microscope. The first signs of clotting are invariably to be noted at the circumference of the drop of blood which is seen to remain at rest while the layer in immediate contact is still freely movable. By moving the drop of blood the edge of the blood may be tested at all points.

The method is extremely sensitive and changes in the behaviour are so easily detected that it is extraordinary how very slight a blast of air is required to detect whether the corpuscles move relatively to one another or no. The degree of force used in blowing and the time during which the blood is moved are the two points of most importance in the use of the apparatus. The blood should be kept as nearly still as possible during the whole time of observation.

In using the apparatus the following precautions are necessary. The surface of the cone which is to pick up the blood must be very thoroughly cleaned. In obtaining the drop of blood, the prick should be fairly large so that the blood will readily come without any pressure. The finger before pricking must be most thoroughly cleansed. We first wash it with soap and water, then with alcohol, and then with ether. The first drop that comes must be discarded, and carefully wiped away, the second drop is then taken. In dipping the glass cone in the blood care must be taken that only the lower surface touches the blood and the whole of this must touch. This ensures the drop being always of approximately the same size. The hanging drop is then brought into the air chamber, the whole of the processes being carried out as rapidly as possible. The position of the whole apparatus on the microscope stage and of the hanging drop relatively to the orifice from which the air issues are then altered until on examining the edge of the drop of blood it is seen that with quite a short and weak current of air the blood is set rotating. If the drop of blood be too large there is at times a difficulty in getting it to rotate at the edge. When all things are properly arranged it is seen that the corpuscles move freely right up to the edge of the drop. At times it is found that at the very edge they remain quiescent, but this is exceptional. In observing the movement it should be noted that they move relatively to one another as well as rotating as a whole, and to be sure of this point it is of course necessary that they be made to move very slowly. If they have been moved out of one position they do not tend to return to that position after the blowing ceases, though if the blowing has been hard enough to markedly indent the surface of the drop they run back to fill up that space when blowing ceases. In this case however they run in from all directions, and not simply backwards from the direction in which the main movement had been caused. As soon as clotting commences however the movements become quite different. The change is always first noted at the free edge and then extends slowly or quickly, depending upon the temperature, to the whole mass. As soon as a rim at the edge is solid, blowing simply indents this rim without causing a rotation. With the indentation there is a certain amount of relative change of position of the superficial corpuscles to the deep, but as soon as blowing ceases they return exactly to their initial position. It is this stage which we always observe, for it is very much more difficult to accurately judge the time at which the whole drop has set solid, without blowing with considerable force. The observation of the edge alone, has the further great advantage that here the layer is thin and the view of the corpuscles is very much better than elsewhere.

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[1] The apparatus is made for us by Mr A. E. Dean, Jun., 73, Hatton Garden, E.C.

The factor in the whole method which we should have liked to avoid is the movement of the blood, but as it is present we attempt to bring any error it may cause down to a minimum by blowing very softly and for only very short intervals of time.

We may now give a few of the results we have obtained upon normal individuals, the times given being those of commencing clotting in minutes and seconds. The first few illustrate the influence of temperature, and are all taken from the same individual. The two at 30° C. were taken on different days. The temperature given is always that of the water circulating through the apparatus.

At 20° C.	At 30° C.[1]	At 30° C.[1]	At 38° C.
(1) 8.0	(1) 3.33	(1) 3.10	(1) 3.49
(2) 8.0	(2) 3.30	(2) 3.0	(2) 3.48
(3) 7.55	(3) 3.30	(3) 3.5	(3) 3.10
(4) 7.42			
(5) 7.46			

The following give a series taken from general results on different individuals.

R. Temp. 20° C.	S.R. Temp. 30° C.	A.M. Temp. 30° C.
(1) 8.40	(1) 3.24	(1) 4.43
(2) 8.18	(2) 3.18	(2) 4.4
(3) 8.13	(3) 3.30	(3) 4.0
		(4) 4.5

W.E.D. Temp. 30° C.	W.S. Temp. 30° C.
(1) 3.0	(1) 3.25
(2) 2.55	(2) 3.40
(3) 3.5	(3) 3.35

In 4 successive drops taken from a vein in the rabbit's ear the times were:

2 min. 13 secs. 2 min. 7 secs. 2 min. 35 secs. 2 min. 16 secs.

the temperature of the circulating water being 30° C.

(Towards the expenses of this research we have received a grant from the Government Grant of the Royal Society.)

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[1] These two sets of observations were taken on two successive days.

THE ACTION OF CERTAIN DRUGS AND MINERAL-WATERS ON THE SECRETION AND COMPOSITION OF HUMAN BILE: AN EXPERIMENTAL INVESTIGATION.[1]

By WILLIAM BAIN, M.D. Durh., M.R.C.P. Lond.

THE investigation of cholagogues is one of the most difficult in the whole domain of pharmacology. The importance of the subject is shown by the number of laborious researches undertaken to determine the action of drugs which are supposed to influence the secretion of bile, and its complexity by the contradictory results obtained. As nearly all recent experimental evidence casts doubt on the existence of so-called cholagogues, it occurred to me that the time would be well spent if I could contribute, even in a minor degree, towards a solution of this very difficult problem. While arrangements were being completed for carrying out a series of investigations on dogs by means of Schiff's amphibolic biliary fistula in the Physiological Laboratory of St Thomas's Hospital, I was informed that a patient with a permanent cutaneous biliary fistula had just left the institution. An interview was immediately arranged with this man, who readily consented to place himself at my disposal for experimental purposes. In mentioning the history of the patient, I wish to acknowledge Dr Payne's kindness in permitting a reference to the notes of the case.

The patient, male, aged 49, was admitted into St Thomas's Hospital, under the care of Dr Payne, on July 19th, 1897, and was operated upon by Mr Anderson on the 29th of the same month. At the operation the gall-bladder was found tensely distended, and contained clear fluid. The cause of the obstruction in the common bile-duct was not ascertained, though a careful search was made. The opinion expressed at the time was that it was probably due to a small impacted gall-stone. As the patient seven months later was in a satisfactory state of health, this view is probably correct. He was discharged from the hospital on October 20th. During his stay there his stools were always clay-coloured. His weight on admission was 9 st.; on his discharge, 8 st. 9 lb.

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[1] From the Physiological Laboratory of St Thomas's Hospital.

When he came under my notice on the 7th of November his health was moderately good. He was anæmic, and troubled more or less with flatulence, but no organic disease could be detected. His appetite was good, and he took a moderate amount of exercise. He lodged close to the hospital, and brought the bile and urine each day to the laboratory. His height was 5 ft. 1 in., and his weight on November 11th, without clothes, 58 kilogrammes (9 st. 2 lb.). He only varied 2 lb. while under my observation, and during the latter part of the period he "felt much better and stronger." His bowels were moved naturally once a day after leaving the hospital, but this action was subsequently modified by the drugs taken. His diet was as follows:—

At 9—Breakfast—Egg, lean bacon, tea and bread.

At 11—Luncheon—One pint of porter.

At 1.30—Dinner—Mutton or beef, potatoes and bread. Occasionally he had fish. Water.

5.30—Tea, bread and butter.

I asked him to take the same amount of food, liquid, and exercise each day, and impressed upon him the necessity for abiding by these rules, as otherwise my results would be vitiated. In addition, he took 36 grains of purified ox-bile daily (12 grains at 10 A.M. and at 2 and 6 P.M.), thus approximating the experimental conditions to the normal standard.

Method of collecting the Bile.—Arbuthnot Lane's biliary tube was employed, which answered the purpose admirably. By means of sticking-plaster the shield was kept in absolute contact with the skin, so that not a drop of bile was lost. The inner was fastened to the outer tube with a small safety-pin to prevent traction. The outer end of the collecting-tube was firmly attached to an india-rubber urinal, fitted with a stopcock. This allowed the patient to collect the bile at stated times without in any way disturbing the connections. The tube was changed about once a week. During the seventy days the case was under observation, only on two occasions was there any oozing, due to an insufficiency of plaster.

The bile was collected four times a day—at 8 A.M., at 12 noon, at 4 P.M., and at 8 P.M.—and put into different sized bottles, properly labelled. The specific gravity of each sample was taken at the laboratory temperature, and the same hydrometer was used throughout. Then the four specimens were put into one bottle, shaken, and a portion reserved for analysis.

METHOD OF ANALYSIS.

Bile Solids.—The weight of the capsule was first ascertained, 25 c.c. of bile measured (the same pipette was always used for this and for estimating the bile salts) and weighed, then evaporated to dryness over a water-bath at a temperature of 100° C. The capsule was next placed in a hot air chamber kept at a temperature of 110° C. by means of a gas regulating apparatus for at least three hours. After that it was placed in an exsiccator until its weight was constant.

Bile Salts.—To 25 c.c. of bile about 120 c.c. of methylated spirit was added, and the whole filtered. The filtrate and washings were evaporated to dryness over a water-bath, and extracted with absolute alcohol. The extract was then evaporated to a small volume, and two-thirds of its volume of anhydrous ether added. This was allowed to stand in a cool place for three days, and the precipitate of bile salts was then collected on a weighed filter. The filtrate was again evaporated to dryness, extracted and precipitated as before, and any further precipitate collected on the same filter. The filter was finally dried at 105 C. to constant weight.

I take this opportunity of expressing my gratitude to Dr Brodie for his advice, and for many acts of kindness. I also have to thank Dr Sikes, Demonstrator of Physiology, for analysing the bile and urine on two occasions during my absence.

For the purpose of comparing results, I will briefly mention the salient facts in the published cases of human biliary fistula.

Monro mentions a case of abscess of the liver where the bile was discharged by coughing, mixed with mucus and saliva. The quantity varied from 10 to 15 oz. in the twenty-four hours. It was irregular in flow, and greater in quantity after dinner.

Charles Robin (2) mentions a case of Taconi's where a patient had a biliary fistula. The bile amounted to 500 grammes in the twenty-four hours.

Harley (3) describes a case of hydatids of the liver where the bile was discharged through a fistulous opening, the quantity varying from 16 to 20 oz.

Murchison (4) records a case of biliary fistula where the bile flowed at the rate of 1 to 2 oz. per hour, usually increasing after meals.

Frerichs (5) quotes a case of Rouis' where 900 grammes of bile a day was expectorated.

Ranke (6) records a case of hydatid disease of the liver where a communication existed between the bile-passages and a bronchus. Sometimes the bile was expectorated, while at others it passed into the duodenum. He gives the results of five observations of twenty-four hours' duration, when the fæces gave no indication of biliary constituents. The patient had chronic bronchitis, and allowance had to be made for the secretion from the respiratory passages. The average of these observations gives 636 c.c. of bile, with 20·6 grammes solid.

Noel (7) mentions a case of biliary fistula. On one occasion 900 grammes of bile were secreted in the twenty-four hours, with a specific gravity of 1·0097.

Von Wittich (8) has published a case where a biliary fistula formed spontaneously on account of an impacted gall-stone. He collected the bile for four hours during the day and for ten hours during the night, and from these data he calculated the amount in the twenty-four hours.

Westphalen (9) describes a case of liver abscess where the bile found its way into the right pleura, and subsequently, after thoracocentesis, through the thoracic wall. Taking the average of seventeen days, the bile amounted to 498 grammes, with 11·268 grammes solid. The bile was analysed by Jacobsen. Westphalen tried the effects of water, calomel, and quinine on the biliary secretion. He found that large quantities of water did not increase the secretion, nor alter the composition of bile. Calomel and quinine had no effect.

Yeo and Herroun (10) made a number of observations on a case of fistula due to cancer of the common duct. The patient was very ill at the time, and took little nourishment. The average quantity of bile secreted during the 24 hours only amounted to 374 c.c., with 5·044 grammes solids. No difference in the rate of secretion was noticed after the small meals taken by the patient.

Hammersten (11) records seven cases of biliary fistula, but in all of them a part of the bile found its way into the intestine. The percentage of solids in his cases varied from 1·63 to 3·53 per cent.

Edington (12) published a case of biliary fistula, but the obstruction was incomplete, so that some of the bile escaped into the duodenum.

The next four cases are important, as the patients, apart from the fistula, were in good health at the time of observation, and the obstruction was complete.

Copeman and Winston (13) record a case of biliary fistula in which the average amount of bile secreted in the 24 hours was 779 c.c., with 11·094 grammes solids. They endeavoured to determine the variations in the flow of bile in relation to time and meals.

Mayo Robson (14) reports some interesting observations which he made on a case of biliary fistula. The quantity of bile collected on various dates during the time the patient was in hospital averaged 850 c.c. Mr Fairley analysed the bile on two occasions: the mean gives 15·28 grammes solids. Mr Robson investigated the action of the following drugs:—calomel, rhubarb, podophyllin, carbonate of soda, iridin, euonymin, turpentine, and benzoate of soda. Unfortunately, Mr Robson did not analyse the bile while these experiments were being carried out; he only gives the total quantities collected. He found that iridin increased the flow temporarily, while aerated soda-water had the same effect, without a subsequent fall. The other drugs had no effect.

Noel Paton and Balfour (15) describe a case of biliary fistula where they made a number of careful observations. They experimented with salicylate of soda, calomel, and ox-bile, but at the time the patient was suffering from a moderately high temperature, so that little importance can be attached to their results. They found that salicylate of soda increased both the quantity and the solids; and that although ox-bile and calomel influenced the secretion, they were less active than the soda salt. A year later, when the patient's health was re-established, Paton (16) had the opportunity of analysing her bile on two successive days. The mean of these observations gave 590 c.c. of bile, with 13·6 grammes solids.

The last recorded case is by Pfaff and Balch (17). Their observations extended over 97 days, and were of a painstaking character. The average of three days without drugs gives 514 c.c., with 8·57 grammes solids. They tried on their patient calomel, corrosive sublimate, salol, ox-bile, human bile, and bile salts. Calomel and corrosive sublimate had no effect; if anything, the quantity was diminished, whereas the percentage of solids did not vary from normal. Salol had some influence, but not pronounced enough to justify them in asserting that it was a cholagogue. Human bile, ox-bile, and bile salts increased both the quantity and the solids.

The amount of Secretion in twenty-four hours.—In taking the average of the daily amount of bile, I excluded those days during which drugs were used, and also the day subsequent to the administration of any drug. Thus the average of sixteen days gave 775 c.c. of bile; the average of nine days 15·893 grammes solids; and the average of 6 days 4·197 grammes bile salts. To save repetition, I may state that when the term *without medication* is used, it refers to these sixteen days. The specific gravity varied during the period without medication from 1·0079 to 1·012. A glance at Table I. will reveal the fact that the quantity of bile in my case is very similar to that of Copeman's and Winston's, but in mine the total solids are much higher. This I attribute to my patient taking ox-bile daily, and also a fair amount of exercise in the open air, while theirs was confined to the wards of an hospital.

[TABLE I.

TABLE I.—(*modified from Gamgee* (18)).—*Exhibiting the Results of different Observers on the Secretion of Bile and Bile Solids (during 24 hours) in the human subject.*

	Ranke.	Von Wittieh.	West-phalen.	Yeo and Herroun.	Copeman and Winston.	Mayo Robson.	Paton.	Pfaff and Balch.	Bain.
Sex.	M.	F.	M.	M.	F.	F.	F.	F.	M.
Weight in kilogrammes,	47	...	...	...	43·7	53	73	51	58
Bile secreted, in c.c., .	636	532	498	374·5	779	819	590	514	775
Bile Solids, in grammes,	20·6	...	11·27	5·04	11·09	15·28	13·596	8·57	15·893
Bile secreted per kilo of body, . . .	13·52 c.c.	...	...	...	17·8 c.c.	16·0 c.c.	8·08 c.c.	10·27 c.c.	13·36 c.c.
Bile Solids secreted per kilo of body, . .	0·44 grms.	...	...	...	0·25 grms.	0·28 grms.	0·186 grms.	0·168 grms.	0·274 grms.

During the period without medication the smallest quantity of bile was secreted on January 20th, 670 c.c., the largest amount on November 11th, 864 c.c.; similarly, the smallest quantity of solids was secreted on January 24th, 15·139 grammes, whilst the largest was on January 11th, 16·640 grammes.

Variations in the rate of Secretion.—There is abundant proof that the rate of secretion is very irregular, but the conditions which operate to produce this irregularity in flow are, to a certain extent, conjectural. The two most potent factors are probably diet and exercise. It is generally admitted that during digestion the secretion of bile is active, but there is a lack of experimental evidence regarding the kind of diet which most influences the secretion. It appears probable that exercise augments the biliary secretion; but if the exercise produces much perspiration, this effect will be annulled. The evidence I have to adduce in support of this statement is exceedingly meagre. On two occasions when the patient was not having drugs, circumstances arose which compelled him to take more than usual exercise. In these two instances the quantity of bile was increased. An average of the days without medication gives the following quantities:—

From 8 to 12 p.m., 147·7 c.c.
„ 12 to 4 p.m., 165·1 c.c.
„ 4 to 8 p.m., 148 c.c.
„ 8 to 8 a.m., 312·6 c.c.

It will be observed there is a rise from 12 to 4, which coincides with the principal meal of the day; and the quantity secreted during the day, 471 c.c., is much larger than that during the night, 312 c.c.

The *colour* of the bile was always of an olive green, the only variation observed being in depth of tint.

The bile was invariably alkaline in reaction.

Pressure of Bile Secretion.—Only one observation was made, on December 14th, at 3.45 p.m., when the pressure was found to be equal to 16 mm. of mercury. This is much less than the mean of Paton's and Balfour's observations, 24 mm. of mercury.

Dr Samuel Fenwick (19) has made numerous observations upon the amount of sulphocyanate of potash in the saliva, in both health and disease. He noticed a marked decrease in the amount when the activity of the nutritional functions was diminished, and in serious febrile affections he regards an increase as an element of favourable prognostic significance. He expresses his belief that the sulphocyanate salt is derived from the biliary salts after they enter the duodenum; and he bases this opinion on the statement that when the bile is prevented from reaching the alimentary canal, as he says happens in one form of dyspepsia characterised by eructations of sulphuretted hydrogen gas, he found the sulphocyanate salt invariably absent from the saliva. If Dr Fenwick's hypothesis be correct, the salt must be absent from the saliva when the common bile-duct is occluded. In order to test this point, the patient was asked to discontinue the ox-bile pills during the Christmas holidays. His saliva was then examined on several occasions, and the sulphocyanate salt was always present, although the quantity was diminished. His saliva was also examined while he was taking ox-bile, but no difference in the reaction could be detected.

In Table II. will be found all particulars relating to the quantity, specific gravity, and analysis both of the bile and urine collected during the 70 days the patient was under my observation.

Table II.—*Exhibiting the Daily Quantity, Specific Gravity, and Analytical Details of the Bile and Urine.*

Date.	Drug.	Hours.	Bile in 24 hours.					
					Solids.		Bile Salts.	
			Quantity in c.cms.	Specific Gravity.	Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1897. November 11th	...	8.12 pm	197	1009	...	...	...	...
		12.4 pm	176	1008				
		4.8 pm	104	10095				
		8.8 am	387	1009				
			864	1·0088				
13th	20 oz. Old Sulphur Well, Harrogate, taken warm at 8 am	8.12 pm	195	1008	...	...	...	...
		12.4 pm	224	1010				
		4.8 pm	142	1008				
		8.8 am	383	1009				
			944	1·009				
14th	...	8.12 pm	187	1009				
		12.4 pm	203	1009				
		4.8 pm	106	1010				
		8.8 am	316	1007				
			812	1·008				
15th	...	8.12 pm	166	1009				
		12.4 pm	173	1009				
		4.8 pm	148	1008				
		8.8 am	360	1008				
			847	1·0083				
16th	Euonymin, 4 grains, taken at 8.30 am	8.12 pm	163	1008				
		12·4 pm	183	1011				
		4.8 pm	143	1007				
		8.8 am	355	10095				
			844	1·0089				
17th	...	8.12 pm	154	1011				
		12.4 pm	167	1011				
		4.8 pm	114	1008				
		8.8 am	275	1009				
			710	1·0097				
18th	Iridin, 4 grains, taken at 8.30 am	8.12 pm	157	1010				
		12.4 pm	190	1010				
		4.8 pm	121	1007				
		8.8 am	312	1009				
			780	1·0091				

URINE in 24 hours.							
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	REMARKS.
...	...	...	...	...	...	...	Patient was 5 feet 1 inch in height.
		Nov. 12	— bile	lost			
...	...	...	...	...	...	...	Patient was 58 kilogrammes in weight, minus clothes.

Date.	Drug.	Hours.	Bile in 24 hours.					
			Quantity in c.cms.	Specific Gravity.	Solids.		Bile Salts.	
					Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1897. November 19th	...	8.12 pm	148	1008				
		12.4 pm	153	1009				
		4.8 pm	131	1007				
		8.8 am	325	1008				
			757	1·0081				
20th	...	8.12 pm	144	1009				
		12.4 pm	165	1012				
		4.8 pm	124	10095				
		8.8 am	285	1010				
			718	1·010				
23rd	...	8.12 pm	147	1008	...	...	...	...
		12.4 pm	183	10085				
		4.8 pm	143	10085				
		8.8 am	312	1009				
			785	1·0086	...	...	3·760	·48
24th	...	8.12 pm	148	1008				
		12.4 pm	162	10095				
		4.8 pm	160	1009				
		8.8 am	325	1008				
			795	1·0085	...	...	3·830	·48
25th	20 oz. Strong Montpellier, Harrogate, taken warm at 8 am	8.12 pm	150	1008				
		12.4 pm	146	10102				
		4.8 pm	148	1009				
		8.8 am	314	10092				
			758	1·0089	...	...	3·820	·50
26th	20 oz. Strong Montpellier, Harrogate, taken warm at 8 am	8.12 pm	142	10085				
		12.4 pm	145	1009				
		4.8 pm	143	10092				
		8.8 am	290	10092				
			720	1·009				
27th	20 oz. Strong Montpellier, Harrogate, taken warm at 8 am	8.12 pm	120	10085				
		12.4 pm	164	10102				
		4.8 pm	145	1009				
		8.8 am	319	1009				
			748	1·0091	...	...	4·011	·53

URINE in 24 hours.							REMARKS.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
...	...	...	...	...	...	...	Not collected on Nov. 21st and 22nd, owing to my absence.

Date.	Drug.	Hours.	Bile in 24 hours.					
					Solids.		Bile Salts.	
			Quantity in c.cms.	Specific Gravity.	Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1897. November 28th	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	138 173 160 339	1009 1009 1009 10085				
			810	1·0088				
29th	20 oz. Kissingen, Harrogate, taken at 8 am	8.12 pm 12.4 pm 4.8 pm 8.8 am	170 181 148 327	1008 1009 10085 10085				
			826	1·0085	16·288	1·95	4·467	·54
30th	20 oz. Kissingen, Harrogate, taken at 8 am	8.12 pm 12.4 pm 4.8 pm 8.8 am	151 212 152 407	10075 10095 10095 10095	...	...	...	...
			922	1·0091	...	...	...	...
December 1st	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	152 180 155 361	1009 10095 1008 1009				
			848	1·0089				
2nd	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	144 163 150 350	10092 1010 1009 10087	...	...	...	...
			807	1·0092	...	...	...	...
3rd	20 oz. Old Sulphur Well, Harrogate, taken warm at 8 am	8.12 pm 12.4 pm 4.8 pm 8.8 am	132 183 169 390	10095 10095 1010 1010				
			874	1·0097	13·427	2·08	...	...
4th	20 oz. Old Sulphur Well, Harrogate, taken warm at 8 am	8.12 pm 12.4 pm 4.8 pm 8.8 am	175 179 162 355	10095 1011 1010 10095				
			871	1·0099	18·650	2·12	5·946	·68

Urine in 24 hours.							Remarks.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
...	...	...	...	...	...	...	No albumen or sugar in the urine.
3275	1008	Faintly Acid	23·605				
...	...	...	...	...	...	...	The urine was examined on this and many subsequent occasions, but no trace of albumen was ever detected.
2524	10105	Acid	19·775				
2164	1011	F. Acid	26·207	·505	·486		
1935	1011	F. Acid	20·170	·510	·421		

Date.	Drug.	Hours.	Bile in 24 hours.					
			Quantity in c.cms.	Specific Gravity.	Solids.		Bile Salts.	
					Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1897. December 5th	20 oz. Old Sulphur Well, Harrogate, taken warm at 8 am	8.12 pm	142	1011	...	...	...	...
		12.4 pm	180	10107				
		4.8 pm	156	1010				
		8.8 am	345	1010				
			823	1·0103	18·675	2·24	5·794	·70
6th	...	8.12 pm	125	10095				
		12.4 pm	195	10085				
		4.8 pm	152	1008				
		8.8 am	351	1008				
			823	1·0083	14·964	1·80	...	...
7th	...	8.12 pm	145	10085				
		12.4 pm	195	10085				
		4.8 pm	163	10085				
		8.8 am	353	1009				
			856	1·0088	16·232	1·88		
8th	20 oz. Soda-Water, taken at 8 am	8.12 pm	160	1009				
		12.4 pm	172	10082				
		4.8 pm	154	1009				
		8.8 am	297	1010				
			783	1·009	16·612	2·10		
9th	...	8.12 pm	144	10095				
		12.4 pm	140	10085				
		4.8 pm	147	1009				
		8.8 am	320	10092				
			751	1·0091	16·246	2·14		
10th	...	8.12 pm	130	1009				
		12.4 pm	171	1008				
		4.8 pm	149	1009				
		8.8 am	292	1009				
			742	1·0088	15·730	2·12	...	...
11th	Benzoate of Soda, 20 grains at 8.15 am	8.12 pm	163	1011				
		12.4 pm	166	1010				
		4.8 pm	181	10095				
		8.8 am	346	10095				
			856	1·0099	18·514	2·16	...	...

Urine in 24 hours.							Remarks.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
...	...	...	...	...	...	...	Patient did not feel well —caught a cold.
2230	1011	F. Acid	19·578	·514	·499		
1602	1011	F. Acid	19·395	·364	·365		
2240	1006	Very F. Acid	13·411	·452	·310		
2000	1009	F. Acid	14·676				
2300	1009	F. Acid	19·993	...	·520		
2071	1008	F. Acid	14·145	...	·317		
1852	1006	Very F. Alkaline	10·154	·254	·238		

Date.	Drug.	Hours.	Bile in 24 hours.					
			Quantity in c.cms.	Specific Gravity.	Solids.		Bile Salts.	
					Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1897. December 12th	...	8.12 pm	147	1009				
		12.4 pm	161	1010				
		4.8 pm	165	10085				
		8.8 am	341	1009				
			814	1·0091	16·444	2·00	...	...
13th	Salicylate of Soda, 15 grains at 8 am, at 1 and at 4 pm	8.12 pm	143	1010	...	...	...	...
		12.4 pm	176	1009				
		4.8 pm	150	1008				
		8.8 am	316	1009				
			785	1·009	15·794	1·87	...	...
14th	...	8.12 pm	152	1009	...	...	...	...
		12.4 pm	173	1010				
		4.8 pm	166	10085				
		8.8 am	300	1009				
			791	1·0091	...	...	...	...
15th	...	8.12 pm	143	1009				
		12.4 pm	165	1010				
		4.8 pm	147	1009				
		8.8 am	304	1009				
			759	1·0001	15·846	2·06	...	...
16th	...	8.12 pm	136	1009				
		12.4 pm	153	1010				
		4.8 pm	164	10095				
		8.8 am	311	10095				
			764	1·0095	16·364	1·99	...	...
17th	...	8.12 pm	148	1011				
		12.4 pm	151	1009				
		4.8 pm	144	1009				
		8.8 am	281	1010				
			724	1·0095	15·292	2·11	...	...
18th	Euonymin, 4 grains at 1 pm, at 5 and at 9 pm	8.12 pm	130	1009	...	...	...	...
		12.4 pm	158	1011				
		4.8 pm	142	1010				
		8.8 am	280	10105				
			710	1·0101	18·580	2·52	5·840	·80

Urine in 24 hours.							Remarks.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
1790	1012	F. Acid	16·146	·431	·381		
...	...	...	...	...	...	...	Leakage from tube, and therefore results not reliable.
2135	1008	Very F. Alkaline	12·774	·611	·262		
...	...	...	...	...	...	...	Bile pressure determined to-day.
2230	1009	F. Acid	16·994	·325	·401		
2150	1008	F. Acid					
1900	1008	F. Acid	20·914	·478	·407		
1880	1009	F. Acid	16·979	·436	·482		
...	...	...	...	...	...	...	Purged six times during the night. Total solids taken from 12 to 12, consequently could not be determined next day.
2200	1004	Neutral	13·660	·688	·421		

Date.	Drug.	Hours.	Bile in 24 hours.					
					Solids.		Bile Salts.	
			Quantity in c.cms.	Specific Gravity.	Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1897. December 19th	Euonymin, 4 grains taken at 8 am	8.12 pm 12.4 pm 4.8 pm 8.8 am	150 133 165 338	1012 1010 10085 1008				
			786	1·0092	...	...	...	...
20th	Euonymin, 2 grs. at 11 am, 2 grs. at 3, and 4 grs. at 9 pm	8.12 pm 12.4 pm 4.8 pm 8.8 am	175 221 170 374	1008 10085 1008 1008				
			940	1·0083	16·015	1·69	4·152	·44
21st	Euonymin, 2 grains taken at 8 am	8.12 pm 12.4 pm 4.8 pm 8.8 am	188 185 166 365	10085 1009 1008 1009				
			904	1·0087	17·314	1·89	5·102	·56
22nd	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	161 167 170 341	10095 1009 1009 1009				
			838	1·0091	16·726	1·99	...	...
23rd	Iridin, 3 grains at 8 am, 12, and 4 pm	8.12 pm 12.4 pm 4.8 pm 8.8 am	143 192 156 310	1010 1010 1009 1010	...	...	...	...
			801	1·0099	...	...	...	...
1898. January 4th	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	lost 174 157 267	 10085 1009 10092				
5th	20 oz. Strong Montpellier, Harrogate, taken warm at 8 am	8.12 pm 12.4 pm 4.8 pm 8.8 am	132 177 151 312	1009 1010 1008 1009				
			772	1·0092	15·312	1·97	3·880	·50

URINE in 24 hours.							REMARKS.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
2197	1005	Faintly Alkaline	10·272	·615	·493		
2420	1007	Neutral	16·111	·655	·439	1·193	
2440	1005	Faintly Alkaline	10·034	·558	·575		
2318	1006	Neutral	11·116	·472	·460	1·130	
...	...	...	...	...	...	...	Solids could not be determined owing to an accident with the bile.
2190	1009	F. Acid	15·713	...	·451	1·097	
2350	1005	F. Acid	10·224	·407	·405	1·478	
2415	1006	F. Acid	13·635	·483	·362	1·0469	

Date.	Drug.	Hours.	Bile in 24 hours.					
			Quantity in c.cms.	Specific Gravity.	Solids.		Bile Salts.	
					Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1898. January 6th	20 oz. Montpellier, Harrogate, at 8 am	8.12 pm	147	1009				
		12.4 pm	172	10095				
		4.8 pm	152	10095				
		8.8 am	270	10095				
			741	1·0094	15·325	2·04	...	...
7th	...	8.12 pm	154	1010	...	...	...	...
		12.4 pm	177	1009				
		4.8 pm	150	1008				
		8.8 am	313	1008				
			794	1·0088	15·463	1·92	3·891	·48
8th	20 oz. Kissingen, Harrogate, at 8 am	8.12 pm	131	1009	...	...	...	...
		12.4 pm	169	1009				
		4.8 pm	146	1010				
		8.8 am	310	1009				
			756	1·0092	15·595	2·04	...	...
9th	20 oz. Kissingen, Harrogate, at 8 am	8.12 pm	126	1010	...	...	...	...
		12.4 pm	228	10075				
		4.8 pm	170	1008				
		8.8 am	355	1009				
			879	1·0085	17·107	1·93	5·002	·56
10th	...	8.12 pm	165	10085				
		12.4 pm	213	10075				
		4.8 pm	167	1008				
		8.8 am	346	10072				
			893	1·0077	15·814	1·77	4·028	·45
11th	...	8.12 pm	162	1008				
		12.4 pm	175	1009				
		4.8 pm	155	1009				
		8.8 am	328	10095				
			820	1·009	16·640	2·03	4·610	·56
12th	20 oz. Chloride of Iron Water, Harrogate, at 8 am	8.12 pm	148	10095				
		12.4 pm	164	10085				
		4.8 pm	163	1009				
		8.8 am	322	1009				
			797	1·0092	16·323	2·02	...	...

Urine in 24 hours.							Remarks.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
1960	1010	F. Acid	15·047	·499	·421		
...	...	...	...	...	...	...	Patient troubled with flatulence.
1934	1009	F. Acid	21·288	·544	·452		
...	...	...	...	...	...	...	Patient had very severe spasms all night.
2025	1006	Neutral	14·238	·583	·376		
...	...	...	...	...	...	...	Patient felt very well to-day.
2600	1005	Neutral	14·530	·589	·370		
2530	1009	F. Acid	13·567	·534	·353	1·2376	
2597	1004	Neutral	9·974	·514	·341	1·433	
2340	1010	Acid	20·077	·589	·449	1·054	

Date.	Drug.	Hours.	Bile in 24 hours.					
			Quantity in c.cms.	Specific Gravity.	Solids.		Bile Salts.	
					Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1898. January 13th	20 oz. Chloride of Iron Water, Harrogate, at 8 am	8.12 pm	136	10085				
		12.4 pm	182	10102				
		4.8 pm	148	1010				
		8.8 am	353	1010				
			819	1·0098	18·305	2·18	5·241	·64
14th	...	8.12 pm	150	1011				
		12.4 pm	240	1009				
		4.8 pm	150	10085				
		8.8 am	324	10095				
			864	1·0094	19·457	2·25	...	...
15th	20 oz. Hot Water, at 8 am	8.12 pm	134	10095				
		12.4 pm	155	1009				
		4.8 pm	157	1009				
		8.8 am	339	1009				
			785	1·0091	16·519	2·09	4·207	·53
16th	...	8.12 pm	150	1009				
		12.4 pm	171	1008				
		4.8 pm	153	1009				
		8.8 am	330	1009				
			804	1·0089	16·400	2·04	...	...
17th	Salicylate of Soda, 15 grains at 9.30 am, at 1 and at 5 pm	8.12 pm	158	10092				
		12.4 pm	171	1009				
		4.8 pm	159	10085				
		8.8 am	355	1009				
			843	1·0089	17·905	2·11	5·462	·68
18th	Salicylate of Soda, 15 grains at 10 am, at 2 and 6 pm	8.12 pm	135	1010	...	...	...	...
		12.4 pm	162	1011				
		4.8 pm	145	1010				
		8.8 am	310	1009				
			752	1·010	16·526	2·17	...	...
19th	...	8.12 pm	142	1009				
		12.4 pm	165	1009				
		4.8 pm	154	1010				
		8.8 am	311	1010				
			772	1·0105	18·311	2·34	5·774	·74

Urine in 24 hours.							Remarks.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
2310	1006	F. Acid	15·661	·549	·435		
2310	1007	F. Acid	16·966	·511	·413	1·0595	
2008	1010	F. Acid	20·874	·553	·428	1·1079	
2215	1008	F. Acid	18·771	·524	·366		
2138	1008	Neutral	15·757	·881	·390		
...	...	...	...	...	...	...	Patient had indigestion.
2177	1010	F. Acid	19·237	·819	·414	1·0825	
2889	1007	Neutral	12·933	·685	·451		

Date.	Drug.	Hours.	Bile in 24 hours.					
			Quantity in c.cms.	Specific Gravity.	Solids.		Bile Salts.	
					Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1898. January 20th	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	134 136 127 273	1010 1011 1013 1010				
			670	1·012	15·304	2·25	3·832	·56
21st	Podophyllo-resin. grain $\frac{1}{5}$ at 1 and 5 and at 8 pm	8.12 pm 12.4 pm 4.8 pm 8.8 am	129 140 158 283	1013 1014 1012 1013				
			710	1·013	18·857	2·63	...	...
22nd	Podophyllo-resin, grain $\frac{1}{5}$ at 8 am, at 12, and at 4 pm	8.12 pm 12.4 pm 4.8 pm 8.8 am	138 165 184 292	1014 1011 1011 1011				
			779	1·0115	18·571	2·34	5·774	·73
23rd	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	132 189 169 327	10105 1010 1009 10095				
			817	1·0095	17·091	2·08	...	...
24th	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	145 157 172 300	1008 10082 10075 1008				
			774	1·0079	15·139	1·98	...	...
25th	Podophyllo-toxin, grain $\frac{1}{5}$ at 8 am, 12, 4 and 8 pm	8.12 pm 12.4 pm 4.8 pm 8.8 am	139 173 152 294	1008 1009 1008 1008	...	...	...	...
			758	1·0082	14·311	1·88	4·044	·56
26th	...	8.12 pm 12.4 pm 4.8 pm 8.8 am	136 137 147 280	10085 10085 10085 1009				
			700	1·0087	13·944	1·99	...	...

URINE in 24 hours.							REMARKS.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
1993	1010	Neutral	12·160	·501	·473		
1908	1012	F. Acid	19·187	·542	·430	1·1736	
1938	1008	F. Acid	17·664	·520	·410	1·0987	
2100	1005	Faintly Alkaline	11·152				
2152	...	Alkaline					
...	...	...	...	...	...	...	The urine was feebly acid when passed; bowels acted twice.
2200	...	Alkaline					
2180	1007	Neutral	15·183	·564	·409		

Date.	Drug.	Hours.	Bile in 24 hours.					
			Quantity in c.cms.	Specific Gravity.	Solids.		Bile Salts.	
					Total in Grammes.	Per cent.	Total in Grammes.	Per cent.
1898. January 27th	...	8.12 pm	140	1009				
		12.4 pm	152	10095				
		4.8 pm	157	10095				
		8.8 am	266	1010				
			715	1·0097	16·016	2·23	4·631	·64
28th	10 oz. Carlsbad at 8 am, and 10 oz. at 8 pm	8.12 pm	139	10095	...	...	...	...
		12.4 pm	163	1010				
		4.8 pm	170	10095				
		8.8 am	321	10105				
			793	1·010	18·312	2·28	5·392	·68
29th	Carlsbad Mineral-Water, 6 oz. at 8 am and 4 oz. at 8 pm	8.12 pm	158	10087				
		12.4 pm	170	10095				
		4.8 pm	174	10095				
		8.8 am	326	10097				
			826	1·0094	18·393	2·20	5·729	·69
30th	...	8.12 pm	146	1007				
		12.4 pm	180	1008				
		4.8 pm	172	10075				
		8.8 am	306	1008				
			804	1·0075	15·362	1·88	4·020	·50
31st	...	8.12 pm	135	10095				
		12.4 pm	165	10095				
		4.8 pm	168	10082				
		8.8 am	285	1009				
			753	1·0089	16·212	2·12	4·520	·60
February 1st	Iridin, 3 grains at 9 am, at 1 and at 5 pm	8.12 pm	139	10095				
		12.4 pm	150	1010				
		4.8 pm	155	1009				
		8.8 am	300	1010				
			744	1·0089	16·849	2·24	5·088	·68
2nd	Iridin, 3 grs. three times a day.	8.12 pm	150	10095				
		12.4 pm	187	1009				
		4.8 pm	184	10095				
		8.8 am	307	1010				
			828	1·0099	18·679	2·47	...	...

Urine in 24 hours.							Remarks.
Quantity in c.cms.	Specific Gravity.	Reaction.	Urea in Grammes.	Uric Acid in Grammes.	Creatinine in Grammes.	Ammonia in Grammes.	
2050	...	Alkaline					
...	...	...	...	...	...	...	Purged seven times during the night.
1900	1009	F. Acid	18·358	·573	·432	1·0489	
2255	...	Alkaline					
2011	1004	Neutral	11·588	·444	·422		
2000	1009	Neutral	14·463	·550	·396		
1910	1010	Neutral	14·138	·534	·439		
1890	1004	Neutral	10·030	·512	·417		

The influence of Drugs on the Secretion of Bile.—Before describing my results it would be advantageous to refer very briefly to the experiments performed on dogs in relation to this subject.

Nasse (20) was the first to investigate cholagogues. He gave one gramme of calomel to a dog with a permanent biliary fistula, and came to the conclusion that calomel increased the quantity of bile, but diminished the bile solids.

Kölliker and Müller (21) tried calomel and aloes without effect.

Mosler (22) and Scott (23) found that calomel did not increase the amount of bile; indeed, the latter said it produced a diminution in the quantity and in the solids. These four experimented on dogs with permanent biliary fistula.

The committee, of which Hughes Bennett (24) was chairman, decided that mercuric chloride, calomel and pil hydrargyri did not increase the flow of bile.

Böhrig (25) experimented on curarised dogs and rabbits. He counted the drops of bile flowing from the canula inserted into the opened gall-bladder,—a method open to criticism. Moreover, he gave such large doses (30 drops of croton oil) that his results must be of little value.

Rutherford's (26) elaborate researches are so well known that a resumé is unnecessary. Rutherford asserts that whatever increases the quantity of bile, similarly affects the solids; and for that reason he did not consider it necessary to estimate the solids in the greater number of his experiments. I maintain that there are drugs which increase the solids without materially affecting the quantity of the bile; therefore it is incumbent upon the investigator to estimate the solids in all his experiments. Further, I consider the total solids are a more important guide to the influence of a drug on the liver than the quantity of bile secreted. Rutherford attaches great importance to the relationship existing between the quantity of bile secreted and the body-weight of the animal. He bases almost all his conclusions upon this supposed relationship—a relationship, however, which is disproved by referring to Table I. Apart from these criticisms, my results, so far as they go, confirm in the main Rutherford's experiments.

Rosenberg (27) used dogs with permanent biliary fistulæ. He found salicylate of soda, olive oil and fats to be cholagogues, the last two being the most powerful. Durand's mixture and Carlsbad salts gave negative results.

Battistini (28) found santonin to be the best cholagogue, and in this is corroborated by Marfori.

Prevost and Binet (30) declared that bile itself was the most powerful cholagogue, but turpentine, chlorate of potash, benzoate and salicylate of soda, salol, euonymin and muscarin were effective. Amongst doubtful substances they placed Carlsbad salts.

Baldi (31) tried podophyllin, rhubarb, jalap, sodium phosphate, pilocarpine, and Carlsbad water without effect, but ox-bile increased the amount and the solids.

Kunkel (32) tried large quantities of water, but the quantity of bile was not increased, nor were the solids affected.

Lewaschew (33) found salicylate of soda effectual.

Paschkis (34) and Niessen (35) obtained negative results with all cholagogues except bile. Since 1890 Stadelmann, and his pupils Niessen, Mandelstamm (36), Müller (37), Lowenton (38), and Glass (39) have conducted a series of careful investigations of cholagogues. Stadelmann (40), summarising the results of these investigations, shows that bile, and possibly salicylate of soda, alone act as cholagogues. He strongly condemns the practice of drawing comparative conclusions regarding the secretion of bile from experiments extending over short periods of time.

Gamgee, commenting on the diversity of these results, suspends his judgment until a fresh research is undertaken on dogs by means of Schiff's amphibolic biliary fistulæ. While I agree with him regarding the value of evidence derived from experiments carried out by Schiff's method, it is my belief that fairly reliable results can be obtained in cases of human biliary fistula by the administration of ox-bile during the entire investigation. Further, although dogs are more easily controlled than human beings as regards food, liquid, and exercise, it is a much more difficult matter in their case to collect the bile without loss.

In Table II. I have drawn up a series of averages, which will help to elucidate my conclusions. Some of the estimations of the bile salts unfortunately miscarried, so that on several occasions the average of bile salts is for a less number of days than those on which a particular drug was given. This also applies in a few instances to the bile solids.

TABLE III.—*Showing the average amount of Bile, Bile Solids, and Bile Salts on the days during which certain Drugs or Mineral-Waters were given.*

Drugs Administered.	Quantity of Bile in c.cms.	Bile Solids.		Bile Salts.	
		Total in grammes.	Per cent.	Total in grammes.	Per cent.
Without Medication,	775	15·893	2·07	4·197	·55
Old Sulphur Spring,	878	18·584	2·14	5·870	·69
Kissengen Spa,	845	16·330	1·97	4·734	·55
Carlsbad Mineral-Water,	809	18·352	2·24	5·560	·68
Euonymin,	836	17·300	2·03	5·031	·60
Benzoate of Soda,	856	18·514	2·16	...	...
Salicylate of Soda,	797	17·215	2·14	5·462	·68
Iridin,	788	17·764	2·35	5·088	·68
Podophyllo-resin,	744	18·714	2·48	7·774	·73
Podophyllo-toxin,	758	14·311	1·88	4·044	·56
Strong Montpellier Spa,	747	15·318	2·00	3·853	·50
Chloride of Iron Spa,	808	17·314	2·10	5·241	·64
Pint of Hot Water,	785	16·519	2·09	4·207	·53
Pint of Soda-Water,	783	16·612	2·10	...	...

The influence of the Harrogate mineral-waters on the secretion of bile has never been tested experimentally; and as I am interested in this particular branch of therapeutics, I began the investigation with them. It should be stated that the waters were sent up to London in bottles, and kept in the laboratory some time before being used.

The Old Sulphur Spring.—The strong sulphur water generally acts as a mild laxative. If the bowels are moved naturally once a day, the only difference it causes, as a rule, is that the evacuations are more copious, and this was the effect upon my patient. An average of four days gave 878 c.c. of bile, with 18·5 grammes solid.

There is no doubt that this water has a very decided effect on the secretion and composition of bile, and I shall show in another paper that this effect is probably due to stimulation of the hepatic cells. As this water contains sulphuretted hydrogen gas, it is possible the effect would have been greater had it been taken at its source.

No other drug or mineral-water with which I experimented produced such a striking increase in both the quantity of bile and bile solids as the Old Sulphur Spring.

Strong Montpellier Spring.—The average of five days shows a diminution in the quantity of bile, in the bile solids, and bile salts; therefore, this water is not a cholagogue. Hunter, in *Allbutt's System of Medicine*, asserts that the beneficial effects of most of the natural mineral-waters are due to a flushing out of the biliary system. In the face of this and other experiments, it is difficult to see how his hypothesis can be maintained.

Kissingen Spa.—The average of four days shows a distinct increase in the quantity of bile, and a slight increase in the solids. The patient had a sharp attack of flatulent dyspepsia one night when he was taking this water, which necessitated the administration of two small doses of brandy. If this day's secretion be omitted, the average rises to 875 c.c. of bile, with 16·6 grammes solids; therefore, the action of the Kissingen water is to increase considerably the quantity of bile without materially augmenting the total solids.

Chloride of Iron Spa.—This water is prescribed for cases of anæmia. It was given to test the alleged effect of iron in reducing the quantity of bile. It should be stated, however, that in addition to iron, it contains a large quantity of chloride of sodium, and appreciable quantities of chlorides of calcium, magnesium, barium, and potash. The dose given was much larger than usual, but it did not disagree. The average of 808 c.c. is somewhat misleading. The day before commencing the water, the quantity of bile was 820 c.c., and the day after it was stopped the quantity rose to 864 c.c. and the solids to

19·457 grammes. It was my intention to have repeated this experiment, but time did not permit. The marked rise, both in the quantity and the solids, on the day after the last administration is a feature of interest.

In regard to the Harrogate waters, it is a point of clinical interest that the relative empirical reputations of the Old Sulphur and the Strong Montpellier Springs are experimentally sustained.

Carlsbad Mineral-Water.—The patient took 10 oz. before breakfast and 10 oz. at bedtime. The second dose purged him seven times. The next day he had 6 oz. before breakfast and 4 oz. at bedtime. The average of the two days gave 808 c.c. of bile, with 18·3 grammes solids. The quantity would be higher were it not for the depletion produced by the purging.

Notwithstanding the negative results obtained by other investigators, I have no hesitation in saying that the high esteem in which this mineral-water is held as a cholagogue is undoubtedly deserved.

A Pint of Hot Water before Breakfast.—This was given on one occasion. The result was 785 c.c. of bile, with 16·5 grammes solids.

A Pint of Soda-Water before Breakfast.—This was also taken on one occasion. The result was 783 c.c. of bile, with 16·6 grammes solids.

In both instances the quantity of bile was scarcely affected, but there was a very slight increase in solids.

Euonymin.—The average of five days gave 836 c.c. of bile, with 17·3 grammes solids; but this does not convey an accurate idea of its effect in relation to the quantity. The patient took 12 grains on the first day, which purged him six times, with a consequent diminution in the quantity of bile, which spoilt the average. It is interesting to note that the solids were the highest on this day. On the last two days the drug was taken the quantity was 940 c.c. and 904 c.c. respectively—a very considerable increase; therefore, euonymin unquestionably increases the quantity of bile, and, to a less extent, the bile solids.

Iridin.—The average of four days gave 788 c.c. of bile, and the average of two days 17·7 grammes solids. From a consideration of these figures, one can safely say that iridin does affect the complex chemical processes which take place in the liver, without increasing the quantity of the bile.

Benzoate of Soda.—This drug was exhibited on one occasion, and in one dose of 20 grains. The result was 856 c.c. of bile, with 18·5 grammes solids. It is undesirable to express an opinion regarding the efficacy of a drug from merely one observation, but I should like to point out that the considerable rise in the total solids can scarcely be regarded as a coincidence, because during the days without medication the solids never exceeded 17 grammes.

Salicylate of Soda.—An average of two days gave 797 c.c. of bile, with 17·2 grammes solids, and the day after the discontinuance of the drug the solids rose to 18·3 grammes; therefore there can be no doubt that it does influence the metabolism of the liver. During its administration the patient suffered a part of one day from indigestion, which might possibly have inhibited the biliary secretion.

With one exception (Niessen), all investigators are agreed that this substance increases the secretion of bile.

Podophylloresin.—This is a resinous subject extracted from Podophyllum Emodi by Professor Dunstan (42), for a sample of which I am indebted to Dr Dixon. It was given in non-purgative doses. The average of two days gave 744 c.c. of bile, with 18·7 grammes solids. This is rather misleading as regards quantity. The day before the drug was given the quantity was only 670 c.c., the first day of administration 710 c.c., the second day 779 c.c., and the day after its discontinuance the quantity rose to 817 c.c., with 17 grammes solids. The increase in the solids is the highest of all substances tested, therefore I think this drug has a decidedly stimulating effect on the liver.

Podophyllotoxin.—This is another extract of Podophyllum Emodi, also extracted by Professor Dunstan (42). It was given on one occasion, and although the doses were supposed to be non-purgative it acted twice on the bowels. The result was 758 c.c. bile, with 14·3 grammes solids, and the day after the solids fell to 13·9 grammes, the lowest during the investigation; consequently, this substance seems to have an adverse influence on the biliary secretion.

Analysis of the Urine.—It was not deemed desirable to ask the patient to bring his urine until he became familiar with collecting his bile. The object in analysing the urine was to get an approximate idea of the amount of nitrogen excreted, and thereby an indication of the quantity of nitrogenous food assimilated. This end could be more accurately attained by adopting some modification of Kjeldahl's process, but I wished to ascertain the action of the drugs used on the excretion of urea and uric acid. Unfortunately, the urine was frequently either neutral or faintly acid, so that conclusions drawn from the results obtained would probably be fallacious. Various expedients were tried to prevent its decomposition, but without much success. The quantity of urine was above the average—about 2000 c.c.—and the specific gravity rarely exceeded 1·010. The colour, considering the quantity, appeared to be normal. Although it was examined repeatedly for albumen, no trace was detected.

For the estimation of urea, Hufner's hypobromite method was employed.

For estimating uric acid, Hopkins' long process was used until January 7th, when I tried Otto Folin's modification of Hopkins' short method. This consists essentially in substituting 10 grammes of ammonium sulphate for saturation with ammonium chloride in a 100 c.c. of urine, and subsequently washing the precipitate with a 10 per cent. solution of ammonium sulphate instead of a saturated solution of ammonium chloride. Folin's method is expeditious, but I do not think it is reliable when the urine is concentrated, and it was found with the dilute urine of my patient that two hours were insufficient for complete precipitation. The uric acid was observed to be increased while the patient was taking salicylate of soda, and, to a slight extent, during the administration of euonymin. Noel Paton found that euonymin increased, while salicylate and benzoate of soda markedly diminished the excretion of uric acid. Hopkins, in Schäfer's *Physiology*, states that the salicylates undoubtedly increase the quantity of uric acid in the urine.

For estimating creatine, Johnson's method was adopted; but a very small amount of the mercurial compound always adhered to the flask, hence a margin of error is introduced into the result.

For the estimation of ammonia, Schloesing's method was adopted; but it is unsatisfactory for clinical purposes, because of the length of time required. As the urea kept persistently below the normal in this case, it was considered probable that the ammonia would be increased in consequence of the diminished formation of urea. Neubauer found the quantity of ammonia to vary in health from ·3 to 1·2 grammes in the twenty-four hours, and this has been confirmed by later observers. In my patient it varied from 1·04 to 1·4 grammes, but it is doubtful whether this slight augmentation is due to the feeble reaction of the urine, or to diminished functional activity of the liver.

SUMMARY OF CONCLUSIONS.

1. The amount of bile secreted in the twenty-four hours in a man somewhat below medium height and weight averages 775 c.c., and the bile solids 15·8 grammes.

2. More bile is secreted during the day than at night.

3. The sulphocyanate of potash in the saliva is not derived from the biliary salts.

4. The following substances increase both the quantity of bile and bile solids:—Old Sulphur Spring (Harrogate), Carlsbad mineral-water, euonymin, benzoate of soda, salicylate of soda, and Kissingen Spa (Harrogate).

5. Podophylloresin and Iridin augment the bile solids without appreciably affecting the quantity of bile.

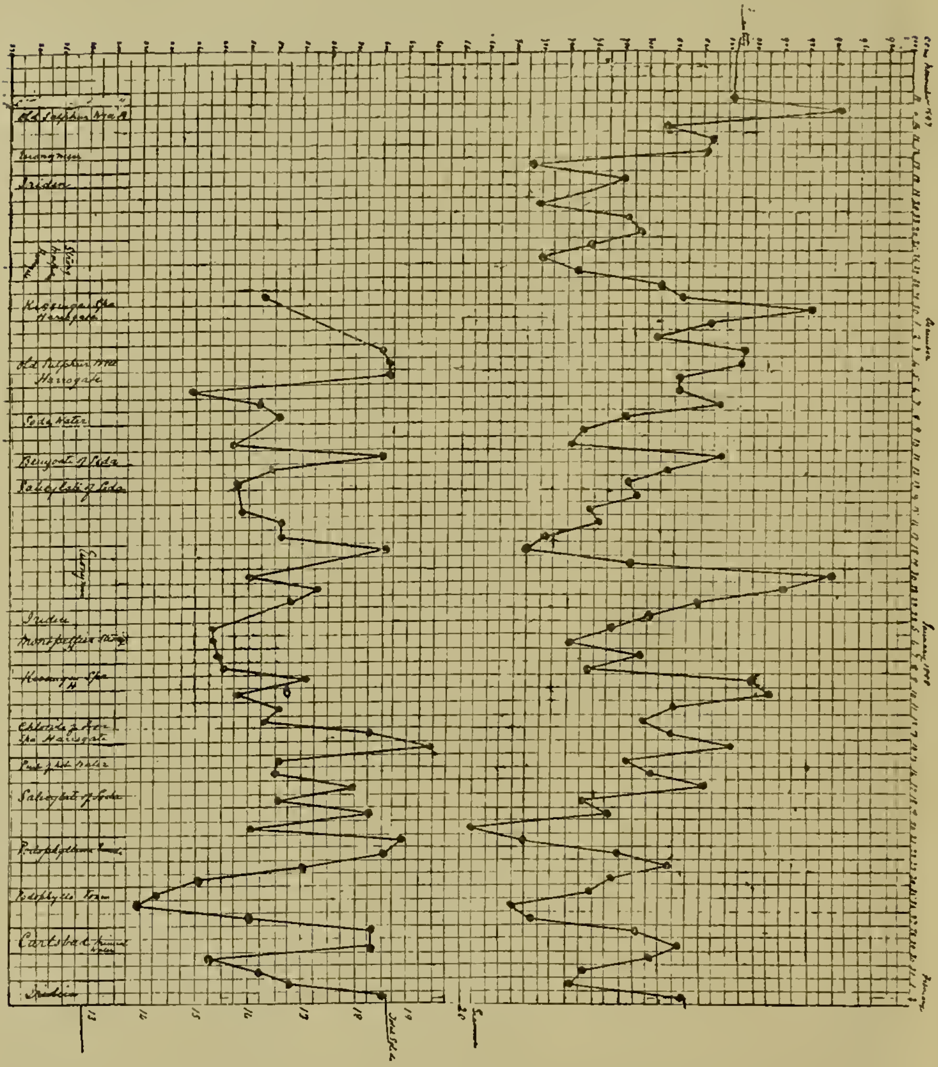
6. Strong Montpellier Spring (Harrogate) and Podophyllotoxin appear to diminish slightly both the quantity and the solids.

7. Hot water and soda-water in pint doses do not seem to increase the biliary secretion.

8. Salicylate of soda increases the excretion of uric acid in the urine.

DESCRIPTION OF THE CHART.

Curves representing graphically the Daily Quantities of Bile and Bile Solids.



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